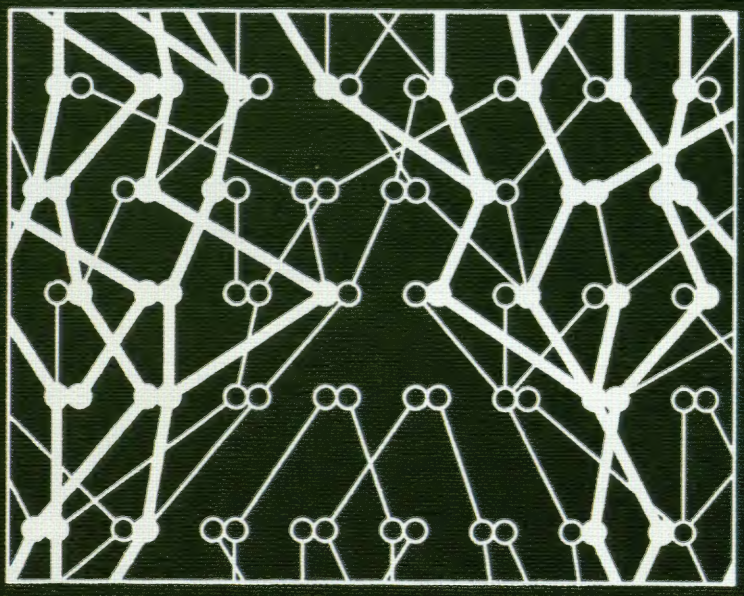
Experimental and Molecular Approaches to Plant Biosystematics



Peter C. Hoch &
A. G. Stephenson

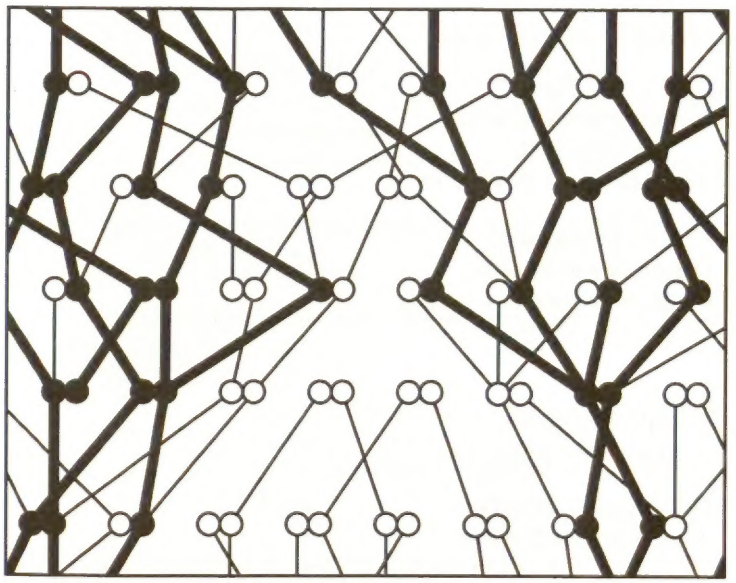
Missouri
Botanical
Garden

Experimental and Molecular Approaches to Plant Biosystematics

Cover illustration adapted from W. Maddison's Figure 4 (p. 278).

REF
QK1
.M833
1993
v.53
c.3

Experimental and Molecular Approaches to Plant Biosystematics



Peter C. Hoch &
A. G. Stephenson

Missouri Botanical Garden

MISSOURI BOTANICAL
GARDEN LIBRARY

MONOGRAPHS IN SYSTEMATIC BOTANY
FROM THE MISSOURI BOTANICAL GARDEN

Volume 53

ISBN 0-915279-30-4
ISSN 0161-1542

AUTHORS' ADDRESSES

Peter C. Hoch:
Missouri Botanical Garden,
P.O. Box 299,
St. Louis, MO 63166-0299, USA

A. G. Stephenson:
Department of Biology,
The Pennsylvania State Universtity,
University Park, PA 16802, USA

SERIES EDITOR
Marshall R. Crosby

MANAGING EDITOR
Amy Scheuler McPherson

EDITORIAL ASSISTANT
Diana Gunter

TEXT FORMATTER
Margaret Overlin

PUBLICATIONS STAFF
Magdalen Lampe
Robin Bruce

Copyright © 1995 by Missouri Botanical Garden.
All rights reserved.

CONTENTS

Preface vii

Contributors ix

Introduction: Perspectives in Biosystematics
Peter C. Hoch and Peter H. Raven xi

I. DNA and Plant Biosystematics

Fossil DNA: Its Potential for Biosystematics
Pamela S. Soltis, Douglas E. Soltis, Stephen J. Novak, Joanna L. Schultz, and Robert K. Kuzoff 1

Nuclear DNA Markers for the Evolution of *Microseris* (Asteraceae)
Konrad Bachmann 15

A Phylogeny of Seed Plants Resulting From Analysis of DNA Sequence Variation Among the *rbc*L Loci of 499 Species, With Particular Emphasis on Alliances Among Monocotyledons
Melvin R. Duvall, Mark W. Chase, Douglas E. Soltis, and Michael T. Clegg 27

Patterns of Transposable Element Evolution in the Grasses
Amy F. MacRae 41

Genetics, Development, and the Morphological Evolution of Maize
John Doebley 57

II. Plant Growth Patterns and Biosystematics

The Fossil History of Branching: Implications for the Phylogeny of Land Plants
Gar W. Rothwell 71

Ecological and Systematic Implications of Branching Patterns in Bryophytes
Michael C. F. Proctor and Anthony J. E. Smith 87

Developmental Themes in Vascular Plants: Functional and Evolutionary Significance
Donald R. Kaplan and Paul A. Groff 111

Distribution of Architectures Across a Taxonomic Spectrum
Francis Hallé 147

Branching Patterns in the Solanaceae
Adrian D. Bell and Trevor D. Dines 157

Plasticity of Branching Patterns in the Clonal Herbs *Trifolium repens* L. and *Glechoma hederacea* L.
Michael J. Hutchings and Roy Turkington 173

III. Plant Reproductive Strategies

Plant Reproductive Strategies: Using the Fossil Record to Unravel Current Issues in Plant Reproduction
David L. Dilcher 187

Genotype-Environment Interaction, Parental Effects, and the Evolution of Plant Reproductive Traits
Johanna Schmitt 199

Gene Flow Among Plant Populations: Evidence From Genetic Markers
J. L. Hamrick, Mary Jo W. Godt, and Susan L. Sherman-Broyles 215

Consequences of Variation in Pollen Load Size
A. G. Stephenson, M. R. Quesada, C. D. Schlichting, and J. A. Winsor 233

Regulation of Flower, Fruit and Seed Production: *Phaseolus coccineus*, A Study Case
O. J. Rocha and A. G. Stephenson 245

Systematics and Reproductive Biology
Gregory J. Anderson 263

IV. Phylogenetic Analysis and Population Biology

Phylogenetic Histories Within and Among Species
Wayne Maddison 273

Genealogical Perspectives on the Species Problem
David A. Baum and Kerry L. Shaw 289

Hybridization and Phylogenetics
Lucinda A. McDade 305

Character Expression, Phylogenetic Reconstruction, and the Detection of Reticulate Evolution
Loren H. Rieseberg and James D. Morefield 333

The Evolution of Self-Incompatibility in Flowering Plants: A Phylogenetic Approach
Stephen G. Weller, Michael J. Donoghue, and Deborah Charlesworth 355

Index 383

Preface

The Fifth International Symposium of the International Organization of Plant Biosystematists (IOPB) was held 11–15 June 1992 in St. Louis, Missouri, with the theme "Experimental and Molecular Approaches to Plant Biosystematics." This triennial meeting of the IOPB drew nearly 300 scientists from around the world for four days of invited and contributed talks and posters, and the opportunity to discuss progress and shared interests in the dynamic field of plant biosystematics.

The 23 papers in this volume were presented as invited lectures in four sessions on each of the mornings of the Symposium. The first session, "DNA and Plant Biosystematics," organized by Barbara Schaal and Michael Clegg, brought together experts doing innovative work with the rapidly developing nucleic acid methods adapted from molecular biology. The papers spanned several levels of organization, including population, species, genus, family, and subclass, and also addressed the analysis of nucleic acids from fossil plants. As amply demonstrated in these and many other contributed papers at the Symposium, it has become possible using appropriate molecular techniques to address many biological questions that biosystematists previously have been able to address only indirectly, if at all. Application of these techniques will continue to be a major focus of biosystematic research.

Adrian Bell organized the second session of invited papers, "Plant Growth Patterns and Biosystematics," around the analysis of plant architecture and branching patterns. A series of broad comparative analyses of patterns in fossil vascular plants, bryophytes, ferns, and angiosperms was followed by analyses of branching patterns in tropical trees, in the plant family Solanaceae, and in several clonal perennial herbs, demonstrating the utility of architectural models in both systematics and ecology.

The third session, "Plant Reproductive Strategies," organized by Andy Stephenson, revisited a subject area familiar to biosystematists with new analytical approaches. Starting again with new insights gained from the study of fossil plants, the papers examined the evolution of reproductive traits, the genetic structure of populations, patterns of pollen flow and deposition, and a specific case study of reproduction in scarlet runner beans. The final paper summarized much that is new in the field, and served as a reminder of the critical importance of reproductive biology in both macro- and micro-evolutionary analyses.

The fourth and final session of the Symposium, organized by Michael Donoghue, addressed the topic, "Phylogenetic Analysis and Population Biology." Whereas the phylogenetic methodology has been applied thus far mainly to addressing relationships among species or higher taxa using morphological or molecular characters, these papers reviewed the application of phylogenetics to population biology and the definition of species, to hybridization and reticulate evolution, and to the evolution of a complex reproductive trait found in many plants, i.e., self-incompatibility.

Taken collectively, the papers of this Symposium, as well as the many contributed papers and posters, reflect the vigor of the biosystematic approach in the 1990s. Theoretical and methodological innovations provide new tools with which to address questions of the definitions, adaptations, and relationships of species. In his opening address reviewing some recent developments in biosystematics, however, Peter Raven also cautioned his audience of the impending crisis of extinction facing the world's biota, particularly in tropical regions, and urged them to select study organisms carefully and to work quickly if we scientists are to have much hope of understanding and documenting the largely unknown tropical biodiversity.

This Fifth International Symposium was organized and sponsored by the IOPB under President Shoichi Kawano, and by the Missouri Botanical Garden, with the assistance of a Steering Committee comprised of Peter H. Raven, Hardy Eshbaugh, Peter Hoch, Sterling Keeley, Meredith Lane, and Barbara Schaal. The details of organizing and actually running the meeting fell to an Organizing Committee of Peter Hoch, Gloria Hoch, and Louise Salmon, the meeting planner for the American Institute of Biological Sciences. To these latter two individuals in particular, I and the IOPB owe a debt of gratitude. The generous financial support of the Mallinckrodt Specialty Chemical Company, the Missouri Botanical Garden, and the Monsanto Company is also gratefully acknowledged.

It is with pleasure and relief that I take this opportunity to thank all of the invited speakers for their excellent contributions, the reviewers of those papers, and all of the other participants in the Symposium, for their efforts and interest that made the St. Louis meeting the great success that it was. Special thanks also go to the Officers and Council of the IOPB for their help and advice in organizing the meeting, and to those staff members and students at the Missouri Botanical Garden who helped in many ways in running the Symposium. For their patient help in editing this volume, I also thank Amy Scheuler McPherson, Managing Editor, and Diana Gunter, both of the Missouri Botanical Garden.

Peter C. Hoch
Missouri Botanical Garden

CONTRIBUTORS

Gregory J. Anderson, Department of Ecology and Evolutionary Biology, U-43, The University of Connecticut, 75 North Eagleville Road, Storrs, Connecticut 06269-3043, USA

Konrad Bachmann, Hugo de Vries Laboratory, Universiteit van Amsterdam, Kruislaan 318, NL 1098 SM Amsterdam, The Netherlands

David Baum, The Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts 02138, USA

Adrian D. Bell, School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW Wales, United Kingdom

Deborah Charlesworth, Department of Ecology and Evolution, University of Chicago, Chicago, Illinois 60637, USA

Mark W. Chase, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, United Kingdom

Michael T. Clegg, Department of Botany and Plant Sciences, University of California, Riverside, Riverside, California 92521, USA

David L. Dilcher, Department of Natural Sciences, Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611-2035, USA

Trevor D. Dines, School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW Wales, United Kingdom

John Doebley, Department of Plant Biology, 220 Bio Sciences Center, University of Minnesota, St. Paul, Minnesota 55108, USA

Michael J. Donoghue, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138, USA

Melvin R. Duvall, Department of Biology & Microbiology, College of Agriculture and Biological Sciences, Box 2207B, South Dakota State University, Brookings, South Dakota 57007-0595, USA

Mary Jo W. Godt, Departments of Botany and Genetics, University of Georgia, Athens, Georgia 30602, USA

Paul A. Groff, Department of Integrative Biology, University of California, Berkeley, Berkeley, California 94720, USA

Francis Hallé, Laboratoire de Botanique, Institute Botanique, 163 rue A. Broussonet, F-3400 Montpellier, France

James L. Hamrick, Departments of Botany and Genetics, University of Georgia, Athens, Georgia 30602, USA

Peter C. Hoch, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, USA

Michael Hutchings, School of Biological Sciences, Biology Building, University of Sussex, Falmer, Brighton, Sussex BN1 9QG, United Kingdom

Donald Kaplan, Department of Plant Biology, University of California, Berkeley, 111 Genetics and Plant Biology Building, Berkeley, California 94720, USA

Robert K. Kuzoff, Department of Botany, Washington State University, Pullman, Washington 99164-4238, USA

Amy MacRae, Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121, USA

Wayne Maddison, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA

Lucinda McDade, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA

James D. Morefield, Nevada Natural Heritage Program, 123 W. Nye Lane, Carson City, Nevada 89710, USA

Stephen J. Novak, Department of Botany, Washington State University, Pullman, Washington 99164-4238, USA

M. C. F. Proctor, Department of Biological Sciences, Prince of Wales Road, University of Exeter, Exeter EX4 4PS, England, United Kingdom

Mauricio R. Quesada, Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

Peter H. Raven, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, USA

Loren H. Rieseberg, Biology Department, Jordan Hall, Indiana University, Bloomington, Indiana 47405, USA

Oscar J. Rocha, Escuela de Biología, Universidad de Costa Rica, Cuidad Universitaria "Rodrigo Facio," San José, Costa Rica

Gar W. Rothwell, Department of Environmental and Plant Biology, Porter Hall, Ohio University, Athens, Ohio 45701-2979, USA

C. D. Schlichting, Department of Ecology and Evolutionary Biology, U-43, The University of Connecticut, 75 North Eagleville Road, Storrs, Connecticut 06269-3043, USA

Johanna Schmitt, Department of Ecology and Evolutionary Biology, Box G-W301, Brown University, Providence, Rhode Island 02912, USA

Joanna L. Schultz, Department of Botany, Washington State University, Pullman, Washington 99164-4238, USA

Kerry L. Shaw, Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, New York 14853-2701, USA

Susan L. Sherman-Broyles, Department of Biological Sciences, SUNY College at Cortland, P.O. Box 2000, Cortland, New York 13405, USA

A. J. E. Smith, School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW Wales, United Kingdom

Douglas E. Soltis, Department of Botany, Washington State University, Pullman, Washington 99164-4238, USA

Pamela Soltis, Department of Botany, Washington State University, Pullman, Washington 99164-4238, USA

Andrew G. Stephenson, Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

Roy Turkington, Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

Stephen G. Weller, Department of Ecology and Evolutionary Biology, School of Biological Sciences, University of California, Irvine, Irvine, California 92717, USA

James A. Winsor, Department of Biology, The Pennsylvania State University, Altoona, Pennsylvania 16603, USA

INTRODUCTION: PERSPECTIVES IN BIOSYSTEMATICS[1]

Peter C. Hoch[2] and Peter H. Raven[2]

ABSTRACT. The field of biosystematics faces major challenges from extrinsic factors. The most critical is the extinction crisis being precipitated by the 20th-century growth in human population, poverty, and overconsumption of natural resources. The second force, whose impact has yet to be fully absorbed by biosystematists, is the computer, and the attendant opportunity for the development of databases. Biosystematics, as well as the entire field of systematics, has been revolutionized by the rapid proliferation of methods for analyzing molecular variation in plants, and by the sheer volume of sequence data now available. This has happened simultaneously with the development of new methods for analysis of systematic data, most notably in cladistics and the statistical methods of numerical taxonomy. Phylogenetic analysis has presented biosystematists with a powerful new tool with which to study evolutionary relationships among taxa. The effect of implementation of new molecular and phylogenetic methods is illustrated using examples from the plant family Onagraceae.

The occasion of the Fifth International Symposium of the International Organization of Plant Biosystematists provides us an opportunity to review the current status of the field of biosystematics and some recent developments in it, and to give some thought to the directions we might want our science and our organization to proceed. We do not intend to provide a history of the field or of the IOPB itself (see Vickery, 1984), but rather to consider selectively some of what we consider to be major issues.

During the past 30 years, two forces have come to shape the broad field of study we call biosystematics, and much else besides. The first of these forces is extinction. Through the diligent work of taxonomists, especially those working with tropical organisms, the number of recognized species has steadily risen, and we are at last beginning to comprehend the true scope of the world's biodiversity (May, 1988; Wilson, 1988; Raven, 1991). Despite earlier estimates that the number of species might be as high as 80 million (Stork, 1988), a widely accepted and more conservative estimate is 5–8 million (May, 1988; Raven, 1991). More than half of these species inhabit the tropics (Wilson, 1988; Raven, 1991).

During these same 30 years, however, we have also begun to comprehend that the modern assault on the environment is driving massive numbers of species to extinction. The explosive growth of the human population to its current level of 5.5 billion, and the conservative estimate that it will reach 8–9 billion within 30 years, exacerbated by grossly unequal distributions of resources and wealth, with the attendant poverty, social injustice, and political instability on the one hand, and non-sustainable overconsumption mainly in the developed world on the other, threatens many of the life-support systems of the planet. Even the most optimistic estimates suggest that at least one-fifth of all species will disappear during this time, and whether the true number of species is 5 million or 30 million (May, 1988; Wilson, 1988), it is clear that a major extinction event is in progress, and that many species will disappear within the coming few decades (Smith et al., 1993).

One obvious result of this extinction process is that systematic biologists increasingly must choose carefully how we will carry out our work. Some two-thirds of all biotic diversity occurs in the tropics, but there is precious little time left in which to study those species. This is especially true for those who would conduct biosystematic studies, since this

[1]We thank the National Science Foundation for support of our work in Onagraceae, most recently as BSR-8906848, and Barbara Schaal, Paul Berry, and Peter Goldblatt for comments on earlier drafts of this paper.

[2]Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

work requires detailed knowledge about chromosome numbers, biochemical and/or molecular constituents, patterns of growth and development, and reproductive biology. The window of opportunity to carry out such important studies on tropical groups of organisms is closing rapidly, and we scientists must act now or lose our remaining chances to learn more about these incredibly diverse and beautiful tropical organisms and communities. Not only must we accelerate our own studies, but we also must act to preserve as much of our biotic diversity as possible in the context of complex and often conflicting societal demands.

The current extinction crisis may impact the science of biology even more severely than I have suggested if E. O. Wilson is correct in his assessment that "The future of biological research lies substantially in the exploration of diversity" (Wilson, 1989). This view suggests that it is precisely the systematic—and biosystematic—approach to the study of organisms, surely enhanced and broadened by the tremendous growth in molecular, cellular, and developmental biology over the past four decades, that will become ascendant in the coming decades as biologists seek the fundamental generalities of biology through the study of diversity.

A second force that has come to shape our endeavor is the computer and the opportunity it presents for the development of databases (Allen, 1993). Implementation of the technology has been slow up to this point, but if systematics is to maintain and expand its utility to society and if we are to keep up with the enormous growth of biological data, we must computerize as much of our information as possible. There is an urgent need for a worldwide relational database, which could free systematists from some of the drudgery of our work and enable us to undertake more important and interesting biosystematic and evolutionary studies. Until we can develop a common database that can be constantly refined and updated, we will waste much time and effort in uncoordinated and repetitive research, at a time when we can ill afford such waste.

There is some danger, however, that inventory work and preparation of botanical databases will be viewed as an end in themselves, ultimately descriptive, simple, and virtually automatic. Nothing, of course, could be further from the truth. Inventory research, a synthetic analysis of all taxa of individual groups found in an area, only provides the basic units that then become the basis for biosystematic and phylogenetic analysis, for information retrieval, and for all further research on those organisms. The ability to sort and recognize units in nature and the resulting classification are critical to the process.

Another, somewhat more intrinsic, change that has strongly influenced the progress of modern biosystematics is the reinterpretation of fossils and of past life that has developed in recent years. As reflected in a number of the papers included in this volume, this new view of past life has resulted from a combination of revealing new collections, novel methods of examining and describing these fossils, and the infusion of new philosophical approaches, stimulated particularly by cladistic methodologies.

BIOSYSTEMATICS 1992

This brings us then to the question: "Where is biosystematics in 1992?" Camp and Gilley (1943) coined the term, but the definition of biosystematics grows and evolves with each new generation of scientists. The functional definition that most of us employ is that it consists of that collection of approaches to systematics that is important and productive for us at any given time and place.

One of the most exciting developments in the past 15–20 years has been the explosive growth of nucleic acid analyses as means to study relationships among organisms as well as rates of evolution within lineages. Along with these advances in technique have come dra-

matic improvements in the ways in which we analyze that new data, as well as the more classical measures of variation, providing us with more robust, consistent, and falsifiable hypotheses. The processes of population differentiation and of speciation itself, the subject of many classical biosystematic studies such as those of Clausen, Keck, and Heisey (1940), have recently been illuminated by genetic analyses that have enabled us to reframe attractive but untestable theories of speciation as testable hypotheses (Coyne, 1992). Overall, our field has won grudging recognition from other branches of science as our work becomes more quantitative and "hard." The remainder of this paper will attempt a very brief review of some recent developments in biosystematics, with emphasis on these exciting new areas, and will consider briefly the example of the plant family Onagraceae to see how our perception of that family has changed in the face of much new work.

Many of the more traditional areas of study in biosystematics remain robust and productive in 1992. Study of breeding systems and reproductive biology generally—the subject of one of the four symposia of the meeting, and sections of this book—continues to provide essential insights into our understanding of population differentiation and speciation. Plant demography in the tradition of Bradshaw (1962), Harper (1977), and others fuses those dynamic results from pollination biology with new results from cytogenetics, ecology, and the growing field of plant growth and development—subject of another of our symposia.

The study of plant chromosomes and their structure, once expected—unrealistically—to provide conclusive insights into the systematics and evolution of all groups, nevertheless has proven to be a powerful tool for the analysis of some taxa at all levels (Stace, 1989). Long-term studies of karyology have provided elegant evolutionary stories in many groups, to be sure. The future holds much promise in this area as the techniques for precise mapping of DNA sequences are combined with new methods of chromosome banding and mapping.

In fact, many so-called traditional approaches to the study of plant biosystematics have been re-invigorated in the past decade by powerful techniques adapted from molecular biology, just as much as has the study of systematics *sensu stricto* (Grant, 1984; Kawano, 1989).

MOLECULAR SYSTEMATICS

Almost since the origin of IOPB as an organization, the application of chemical data to the study of plants has been one of the most active areas in the discipline of biosystematics (Fairbrothers et al., 1975; Vickery, 1984; Crawford, 1985). The emphasis in plant chemosystematics has changed during the past 20 years from the more accessible secondary compounds like flavonoids and terpenoids to proteins, and finally to nucleic acids. The study of secondary metabolites became a major systematic tool with the development of powerful new techniques such as chromatography and electrophoresis, and their successful application in the work of Alston and Turner (1963) on *Baptisia*, and of Swain (1963), Harborne (1970), and many others, but it gradually became clear that more immediate products—proteins and nucleic acids—could provide more precise and consistent evolutionary markers at virtually all levels of biological organization. As early as 1967, Alston suggested that "the future seems to belong to the macromolecules" (Alston, 1967). Even though recent studies of secondary compounds still contribute insights to systematic biology, a quick glance through the program for this meeting demonstrates how valid was his perception, perhaps even beyond his expectations.

Even in the 1960s, it was clear that the newly expanding field of molecular biology could revolutionize biosystematics. In 1967, the same year in which Alston made his predic-

tion, Fitch and Margoliash (1967) reported their pioneering amino acid sequencing work, which ushered in this new approach. The study of proteins, the products directly encoded by DNA, promised to provide much more precise data about variation and evolution in plants, and even perhaps a "true" phylogeny (Boulter et al., 1972). Allozyme analyses using electrophoresis by Gottlieb (1973, 1977) and many others provided indirect estimates of gene flow in plant populations, evidence of hybridization and the origin of polyploids, and other powerful data on population dynamics. Protein studies in general have had less utility above the specific level, and with some exceptions (e.g., the Rubisco amino acid sequence analyses of Martin and Dowd [1984, 1986]) have not provided much data for phylogenetic analyses. If the promise placed in protein analysis as a systematic tool has not been fully realized, it may be due primarily to the tremendous growth in nucleic acid methods. Indeed, new techniques using DNA sequencing and RAPD (random amplified polymorphic DNA) analysis may soon supplant allozymes at the population level because they provide more specific genotype markers, and can be used to determine paternity and measure overall levels of variation, analyze introgression and hybridization, map quantitative trait loci, and study diversification of gene lineages.

One needs to look no farther than the program for this meeting to see the degree to which nucleic acid analyses have become one of the primary tools for systematic research. Slightly more than half of all contributed presentations to this symposium derive their results in whole or in part from study of nucleic acids, and many others include analyses of other chemical or molecular constituents. Following the early work of zoologists like Wilson, Goodman, Lewontin, and others, "molecular botany" developed through the work of Chase, Clegg, Doyle, Palmer, Schaal, the Soltises, Sytsma, White, Zimmer, and now many second- and third-generation students of their innovative work (Crawford, 1990; Olmstead and Palmer, 1994; Sytsma and Hahn, 1994).

Evolutionary questions at any taxonomic level can be addressed with a DNA sequence, but great care must be taken to select the right one. Restriction fragment and/or sequence analysis of nuclear ribosomal DNA (rDNA) has proven very useful, at the population level using the highly variable intergenic spacing region (Schaal and Learn, 1988), at the generic level (Sytsma and Schaal, 1985, in *Lisianthius*), and at intra-familial (Hamby and Zimmer, 1988, in Poaceae) and inter-familial levels of organization (Nickrent and Franchina, 1990, in Santalales), using the more conservative 18S and 26S rRNAs. The development of polymerase chain reaction (PCR)-based methods of sequencing nucleic acids has provided extremely powerful and precise information on the phylogenetic relationships among taxa, even in the hands of non-molecular biologists, and sequence data increasingly will be the basis on which the evolution of molecular and phenotypic characters is studied (Holzman, 1992; Penny et al., 1992).

Chloroplast DNA (cpDNA) has been used more extensively than other molecules, largely because cpDNA is of relatively small and nearly uniform size (about 150 kb), and of relatively well-known structure (Olmstead and Palmer, 1994; Sytsma and Hahn, 1994). On average, the chloroplast genome is highly conserved, more so than most nuclear DNA. Different parts of the cpDNA molecule can be used to compare congeneric species (Palmer et al., 1988), at the subtribal level (as in Asteraceae; Jansen and Palmer, 1988), the generic level (Sytsma et al., 1990, in *Clarkia*), and even among conspecific populations (Whittemore & Schaal, 1991). Most systematic research using the chloroplast genome has involved two slowly evolving sequences (*rbc*L and rRNA genes). An entire recent issue of the *Annals of the Missouri Botanical Garden* (vol. 80, no. 3, 1993) was devoted to results of the coordinated analysis of *rbc*L in plants. The molecular phylogenies are increasingly able to address questions of "deep" phylogeny, such as the origin of the monocots, and relationships among

and between orders. One rather new aspect of plant evolution made clear by recent studies of rates of molecular evolution is that some plant lineages and families evolve more rapidly than others (Sytsma and Hahn, 1994).

Another extremely exciting new area of biosystematics is the analysis of fossil DNA sequences using PCR-mediated techniques (Holzman, 1992; Soltis et al., 1995 this volume). This remarkable work will enable us to determine relationships of extinct and extant plants and to calibrate rates of evolution in those lineages.

The development of appropriate methods of statistical analysis of variation in nucleic acid sequences, fragment lengths, and restriction sites has been crucial to the development of the molecular techniques, since it provides a measure of statistical confidence in the results. This is particularly important as we progressively confront phylogenies derived from different sources of data, and need some indication of the relative consistency of each, as well as methods for determining degrees of congruence among them.

One final aspect of the molecular work that should be mentioned is the possibility of reanalyzing aspects of morphological evolution in a particular group by starting from a phylogeny derived from molecular data. In this way it is possible to study direction and speed of evolution of certain characters, or to analyze more precisely, for example, how pollination syndromes in different taxa arose, without the danger of circular reasoning.

PHYLOGENETIC ANALYSIS

The earliest attempt to classify organisms based on overall similarity using numerous equally weighted characters was that of the French botanist Adanson (1763). His efforts were widely derided and condemned, like those of his successors some 200 years later, but unlike their efforts, his were quickly forgotten! In fact, in the early 1960s two new approaches to phylogeny appeared. Phenetics, or numerical taxonomy as it was originally called by Sneath (1957), Cain (1962), Sokal, Rohlf and others (Sokal and Sneath, 1963; Sokal et al., 1965; Sneath and Sokal, 1973), argued that phylogeny was unknown and largely unknowable. They proposed objective and repeatable procedures to evaluate similarities between organisms (or OTUs), without regard to questions of descent. All characters were equally weighted, as many characters as possible compared, and relationships expressed as an index of similarity. The statistical and mathematical methods of this approach have been widely accepted and adapted to many uses, both in biology and elsewhere, but as a theoretical approach to systematics, phenetics has relatively fewer followers than the second method that arose almost simultaneously.

Cladistics, which makes a distinction between overall similarity and derived similarity, arose from the conviction that procedures to permit genealogical classification must be found. The principles of cladistics were developed by the German entomologist Willi Hennig in 1950, but his work did not begin to reach a broad audience of biologists until 1966, when the English edition of his book *Phylogenetic Systematics* appeared (Hennig, 1966). A critical factor in the acceptance of Hennig's ideas was the nearly simultaneous appearance in 1966 of Brundin's (1966) now-classic monograph on transantarctic midges, which strongly impressed Gareth Nelson of the American Museum of Natural History, an institution that went on to become a bastion of the cladistic method in the ensuing struggle of ideas.

The most basic principles of Hennig's theory of cladistics include: 1) relationship is clearly defined in terms of recency of common ancestry; 2) relationship is detected by means of shared derived characters (synapomorphies), which are homologies that characterize monophyletic groups; 3) relationships and character distributions can be expressed in dichotomous branching diagrams (cladograms); and 4) only natural groups, defined by syna-

pomorphies to be monophyletic (i.e., descended from a common ancestor), are to be admitted into Linnaean classification schemes. Arguments about all of these basic ideas have raged between the cladists, led by Nelson, Farris (1970), and many others, and the pheneticists on one hand, and the "evolutionary" systematists like Mayr and Simpson on the other. To a large degree, the cladistic approach has now become widely accepted within the systematic community as the primary basis for describing evolutionary relationships. Cladistics makes no special conditions for source of data; it is possible to reconstruct the history of groups of any kind, with any mixture of fossil and living taxa, using any mixture of morphological and molecular data.

Cladistics has also contributed to a resurgence of activity and interest in biogeography, or at least in the sub-discipline of historical biogeography, which seeks explanations for the patterns of geographical distribution of organisms in space through time, based on the physical history of the earth (Nelson and Platnick, 1981; Cracraft, 1988; Crisci et al., 1991). In fact, the field of historical biogeography is currently embroiled in a spirited contest of ideas and methods to explain observed patterns, which pits the traditional dispersalist explanation of Simpson (1965), and others, against panbiogeography (Croizat, 1964; Craw, 1989) and cladistic biogeography (Nelson and Platnick, 1981; Page, 1988). Each method provides useful analyses and insights, but none offers an explanation of all patterns and questions in the field (Crisci et al., 1991).

ONAGRACEAE AS AN EXAMPLE

The plant family Onagraceae provides a good example of an intensively studied group in which to examine the influence of the new analyses available through molecular systematics and phylogenetics. The basic taxonomy of Onagraceae (7 tribes, 16 genera, about 674 species) has been established by a series of monographic studies starting with Lewis and Lewis's (1955) monograph of *Clarkia*, and recently summarized by Raven (1979, 1988) and Hoch et al. (1993a). Classical systematic analysis of the phylogeny of Onagraceae (Raven, 1988) has been based on detailed morphological information from floral, leaf, and wood anatomy, embryology, and palynology, on knowledge of chromosome numbers, morphology, and (in some groups) structure, on a nearly complete survey of flavonoids and, to a lesser extent, other micromolecules, and on extensive data regarding pollination biology, compatibility relationships, and other aspects of reproductive biology (summarized in Raven, 1979, 1988; Hoch et al., 1993a).

The first nucleic acid analysis in Onagraceae was initiated by Les Gottlieb and Ken Sytsma on cpDNA restriction site variation in *Clarkia* (summarized in Sytsma et al., 1990; Sytsma and Smith, 1992). An unexpected result from this analysis (Sytsma and Gottlieb, 1986) was the hypothesis that the distinctive monotypic genus *Heterogaura* originated within *Clarkia* section *Sympherica*, one of the most advanced sections of the genus (Lewis and Lewis, 1955; Sytsma and Smith, 1992), and is most closely related to *C. dudleyana*. Raven (1964) earlier had suggested that *Heterogaura* was more closely related to *Clarkia* than to any other genus, but its striking morphological differences precluded placing it within *Clarkia*. Although *Heterogaura* is now formally included in *Clarkia* (Lewis and Raven, 1992), the analysis of the morphological evolution of the entire genus, and particularly the remarkable morphological transformation that has taken place in the one species (the former *Heterogaura*), is not yet complete.

Even with the tremendous amount of detailed information on Onagraceae, it was possible to make very few clear hypotheses about the relationships of the major tribal lineages of the family, although it is very well delimited and clearly belonging to the order Myrtales (Dahlgren and Thorne, 1984; Tobe and Raven, 1983; Raven, 1988). The strongest data came

from floral anatomy (Eyde, 1977, 1979, 1981), which established that *Ludwigia* was the sister group of the rest of the family. Morphological characters from various sources and addressing certain other relationships often were contradictory. A cladistic analysis of the 16 genera of the family (Hoch et al., 1993a) based on 17 non-molecular characters produced seven equally parsimonious trees, and the topology in the strict consensus tree suggested several hypotheses about relationships among other tribal lineages. One of these hypotheses was the possibility that the large tribe Onagreae is not monophyletic, because *Gayophytum*, a small annual genus unambiguously included in previous circumscriptions of Onagreae (Raven, 1979), is most closely related to *Epilobium* (tribe Epilobieae), and because the enigmatic genus *Hauya* (tribe Hauyeae) appeared nested within an advanced lineage of Onagreae.

Analysis of variation in nucleic acids in Onagraceae has been pursued concurrently in collaboration with Ken Sytsma and his colleagues in Madison using cpDNA restriction site variation (Sytsma et al., 1991) and *rbc*L sequences (Conti et al., 1993), and with Bult and Zimmer (1993; using nuclear rDNA sequence) in Washington, D.C. Two additional analyses of tribal relationships are also available, based on *rbc*S amino acid sequence data (Martin and Dowd, 1986) and rDNA restriction site variation (Crisci et al., 1990). Conti et al. (1993) compared topologies of the consensus trees of these five molecular analyses, plus that from the morphological study, including the number of extra steps required to force the *rbc*L data onto the topologies generated from the other five data sets (Figure 1). The cpDNA restriction site tree was most similar to the *rbc*L tree (one step longer), and the morphology tree least similar (14 steps longer). None of the six trees resulting from these analyses is identical to any other in proposing relationships of the major lineages in the family, with the main differences involving the position of *Lopezia* and *Hauya*. The diversity of hypotheses is somewhat unsettling; only a single hypothesis is consistent throughout—that *Ludwigia* is a sister group to the rest of the family. Despite the lack of agreement, it is probable that tribes Onagreae and Epilobieae form a monophyletic group (although the structure within that clade is not yet clear [Baum et al., 1994]), and that *Circaea*, *Fuchsia*, and possibly *Hauya* form another monophyletic branch. The position of *Lopezia* remains uncertain; the two chloroplast studies place it basal to the Onagreae-Epilobieae clade, but without much statistical support (Conti et al., 1993). An analysis of these six data sets, possibly with a seventh based on nrDNA ITS (internal transcribed spacer) sequence data (Baum et al., unpublished), is now in progress that will correct for missing taxa and other inconsistencies among the analyses, and will use both total evidence and congruence approaches in an effort to achieve a statistically robust reconstruction of the family.

The more precise methods of phylogenetic analysis and the veritable flood of molecular systematic data provide explicit hypotheses regarding relationships in Onagraceae, suggesting further studies to elucidate the evolution of the family. For example, Steve O'Kane, working with Barbara Schaal, has analyzed *Lopezia* using this cpDNA restriction site and nrDNA ITS sequence data (O'Kane, in prep.). The resulting molecular phylogeny can be compared to the morphological hypothesis of relationships in the genus (Plitmann et al., 1973; Hoch et al., 1993b), and used to analyze the evolution of the complex chromosomal evolution of the genus, its remarkable diversity of pollination syndromes, and its biogeography (Plitmann et al., 1973; Raven, 1979).

This capability to use independently derived phylogenetic hypotheses to examine morphological and ecological aspects of evolution is one of the most exciting opportunities available to biosystematists. Let us hope that collectively we can bring these powerful new techniques to bear on many groups of tropical plants, so that we may reconstruct the unique evolutionary histories of these endangered organisms and learn as much about them as possible before they are gone.

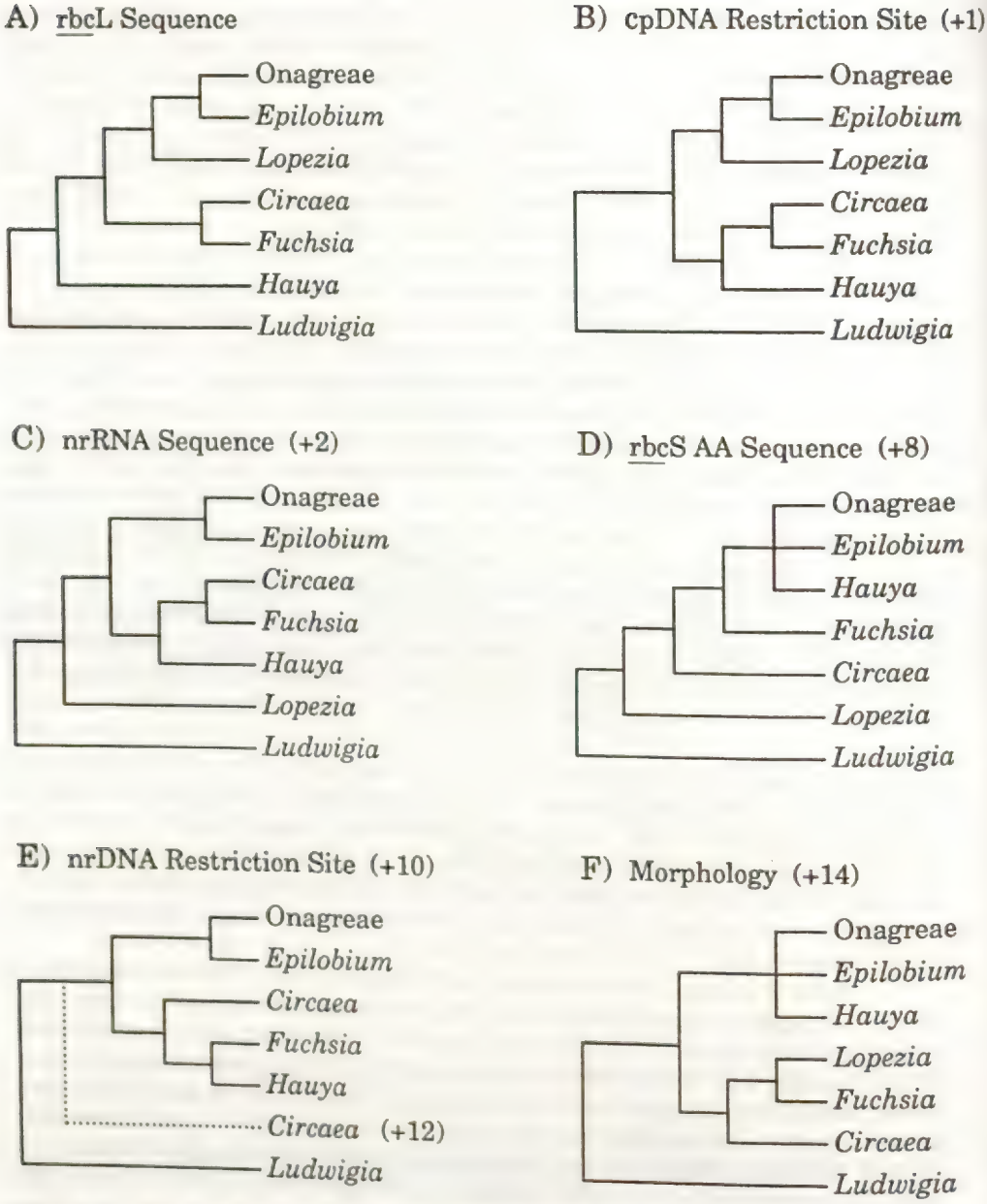


Figure 1. Reprinted from Conti et al. (1993) with permission. Topologies of the consensus trees from six cladistic analyses of various data sets for Onagracae, modified to include a comparable set of taxa. The number of extra steps required to force the *rbc*L sequence data onto topologies from the other data sets is indicated in parentheses. In Figure 1E (which did not include *Lopezia*), *Circaea* is shown in two alternative positions: the solid line indicates its placement in the Dollo parsimony, 50% majority rule consensus tree; the dotted line shows it placement in the Wagner parsimony, 50% majority rule consensus tree. The trees were drawn based on the following original analyses: A. Conti et al. (1993); B. Sytsma et al. (1991); C. Bult and Zimmer (1993); D. Martin and Dowd (1986); E. Crisci et al. (1990); and F. Hoch et al. (1993a).

LITERATURE CITED

Adanson, M. 1763. Familles des Plantes. Vol. 1. Vincent, Paris.

Allen, W. H. 1993. The rise of the botanical database. Bioscience 43: 274–279.

Alston, R. E. 1967. Biochemical systematics. Evol. Biol. 1: 197–385.

_____and B. L. Turner. 1963. Natural hybridization among four species of *Baptisia* (Leguminosae). Amer. J. Bot. 50: 159–173.

Baum, D. A., K. J. Sytsma, and P. C. Hoch. 1994. The phylogeny of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. Syst. Bot. 19: 363–388.

Boulter, D., J. A. Ramshaw, E. W. Thompson, M. Richardson, and R. H. Brown. 1972. A phylogeny of higher plants based on the amino acid sequence of cytochrome c and its biological implications. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 181: 441–455.

Bradshaw, A. D. 1962. The taxonomic problems of local geographical variation in plant species. Pp. 7–16 *in* D. Nichols (ed.), Taxonomy and Geography. Systematics Association, London.

Brundin, L. 1966. Transantarctic relationships and their significance, as evidenced by chironomid midges. Kung. Svenska Vetenskapsakad. Handlingar 11: 1–472.

Bult, C. J. and E. A. Zimmer. 1993. Nuclear ribosomal RNA sequences for inferring tribal relationships within Onagraceae. Syst. Bot. 18: 48–63.

Cain, A. J. 1962. The evolution of taxonomic principles. Pp. 1–13 *in* G. C. Ainsworth and P. H. A. Sneath (eds.), Microbial Classification. Cambridge University Press, New York.

Camp, W. H. and C. L. Gilley. 1943. The structure and origin of species. Brittonia 4: 323–385.

Clausen, J., D. D. Keck, and W. M. Heisey. 1940. Experimental studies on the nature of species, I. The effect of varied environments on western North American plants. Publ. Carnegie Inst. Wash. 520: 1–452.

Conti, E., A. Fischbach, and K. J. Sytsma. 1993. Tribal relationships in Onagraceae: implications from *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 672–685.

Coyne, J. A. 1992. Genetics and speciation. Nature 355: 511–515.

Cracraft, J. 1988. Deep-history biogeography: retrieving the historical pattern of evolving continental biotas. Syst. Zool. 37: 221–236.

Craw, R. C. 1989. Quantitative panbiogeography: introduction to methods. New Zealand J. Zool. 16: 485–494.

Crawford, D. J. 1985. Electrophoretic data and plant speciation. Syst. Bot. 10: 405–416.

_____. 1990. Plant Molecular Systematics: Macromolecular Approaches. John Wiley and Sons, New York.

Crisci, J. V., M. M. Cigliano, J. J. Morrone, and S. Roig Juñent. 1991. A comparative review of cladistic biogeography approaches to historical biogeography of southern South America. Austral. Syst. Bot. 4: 117–126.

_____, E. A. Zimmer, P. C. Hoch, G. B. Johnson, C. Mudd, and N. Pan. 1990. Phylogenetic implications of ribosomal DNA restriction site variation in the plant family Onagraceae. Ann. Missouri Bot. Gard. 77: 523–538.

Croizat, L. 1964. Space, Time, Form: the Biological Synthesis. Published by the author, Caracas.

Dahlgren, R. and R. F. Thorne. 1984. The order Myrtales: circumscription, variation, and relationships. Ann. Missouri Bot. Gard. 71: 633–699.

Eyde, R. H. 1977. Reproductive structures and evolution in *Ludwigia* (Onagraceae). I. Androecium, placentation, merism. Ann. Missouri Bot. Gard. 64: 644–655.

_____. 1979. Reproductive structures and evolution in *Ludwigia* (Onagraceae). II. Fruit and seed. Ann. Missouri Bot. Gard. 66: 656–675.

_____. 1981. Reproductive structures and evolution in *Ludwigia* (Onagraceae). III. Vasculature, nectaries, conclusions. Ann. Missouri Bot. Gard. 68: 379–412.

Fairbrothers, D. E., T. J. Mabry, R. L. Scogin, and B. L. Turner. 1975. The bases of angiosperm phylogeny: Chemotaxonomy. Ann. Missouri Bot. Gard. 62: 765–800.

Farris, J. S. 1970. Methods for computing Wagner Trees. Syst. Zool. 19: 83–92.

Fitch, W. M. and E. Margoliash. 1967. Construction of phylogenetic trees. Science 155: 279–284.

Gottlieb, L. D. 1973. Enzyme differentiation and phylogeny in *Clarkia franciscana*, *C. rubicunda*, and *C. amoena*. Evolution 27: 205–214.

_____. 1977. Electrophoretic evidence and plant systematics. Ann. Missouri Bot. Gard. 64: 161–180.

Grant, W. F. (ed.) 1984. Plant Biosystematics. Academic Press, New York.

Hamby, R. K. and E. A. Zimmer. 1988. Ribosomal RNA sequences for inferring phylogeny within the grass family. Plant Syst. Evol. 160: 29–37.

Harborne, J. B. (ed.) 1970. Phytochemical Phylogeny. Academic Press, London.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, London.

Hennig, W. 1966. Phylogenetic Systematics. University of Illinois Press, Urbana.

Hoch, P. C., J. V. Crisci, H. Tobe, and P. E. Berry. 1993a. A cladistic analysis of the plant family Onagraceae. Syst. Bot. 18: 31–47.

_____, J. V. Crisci, and H. Tobe. 1993b. A cladistic analysis of the genus *Lopezia* (Onagraceae). Bot. J. Linn. Soc. 111: 103–116.

Holzman, D. 1992. A tool for all seasons. Mosaic 23: 2–11.

Jansen, R. K. and J. D. Palmer. 1988. Phylogenetic implications of chloroplast DNA restriction site variation in the Mutisieae (Asteraceae). Amer. J. Bot. 75: 753–766.

Kawano, S. (ed.) 1989. Biological Approaches and Evolutionary Trends in Plants. Academic Press, London.

Lewis, H. and M. E. Lewis. 1955. The genus *Clarkia*. Univ. Calif. Publ. Bot. 20: 241–392.

_____ and P. H. Raven. 1992. New combinations in the genus *Clarkia* (Onagraceae). Madroño 39: 163–169.

Martin, P. G. and J. M. Dowd. 1984. The study of plant phylogeny using amino acid sequences of ribulose-1,5-bisphosphate carboxylase. IV. Proteaceae and Fagaceae and the rate of evolution of the small subunit. Austral. J. Bot. 32: 291–299.

_____ and _____. 1986. Phylogenetic studies using protein sequences within the order Myrtales. Ann. Missouri Bot. Gard. 73: 442–448.

May, R. M. 1988. How many species are there on Earth? Science 241: 1441–1449.

Nelson, G. J. and N. I. Platnick. 1981. Systematics and Biogeography, Cladistics and Vicariance. Columbia University Press, New York.

Nickrent, D. L. and C. R. Franchina. 1990. Phylogenetic relationships of the Santalales and relatives. J. Molec. Evol. 31: 294–301.

Olmstead, R. G. and J. D. Palmer. 1994. Chloroplast DNA systematics: a review of methods and data analysis. Amer. J. Bot. 81: 1205–1224.

Page, R. D. M. 1988. Quantitative cladistic biogeography: constructing and comparing area cladograms. Syst. Zool. 37: 254–270.

Palmer, J. D., R. K. Jansen, H. J. Michaels, M. W. Chase, and J. R. Manhart. 1988. Chloroplast DNA variation and plant phylogeny. Ann. Missouri Bot. Gard. 75: 1180–1206.

Penny, D., M. D. Hendy, and M. A. Steel. 1992. Progress with methods for constructing evolutionary trees. Trends Ecol. Evol. 7: 73–79.

Plitmann, U., P. H. Raven, and D. E. Breedlove. 1973. The systematics of Lopezieae (Onagraceae). Ann. Missouri Bot. Gard. 60: 478–563.

Raven, P. H. 1964. The generic subdivision of Onagraceae, tribe Onagreae. Brittonia 16: 276–288.

_____. 1979. A survey of reproductive biology in Onagraceae. New Zealand J. Bot. 17: 575–593.

_____. 1988. Onagraceae as a model of plant evolution. Pp. 85–107 *in* L. D. Gottlieb and S. K. Jain (eds.), Plant Evolutionary Biology. A symposium honoring G. Ledyard Stebbins. Chapman and Hall, London.

_____. 1991. Winners and losers in the twentieth-century struggle to survive. Pp. 259–267 *in* K. Davis and M. S. Bernstam (eds.), Resources, Environment, and Population: Present Knowledge, Future Options. Oxford University Press, New York.

Schaal, B. A. and G. H. Learn, Jr. 1988. Ribosomal DNA variation within and among plant populations. Ann. Missouri Bot. Gard. 75: 1207–1216.

Simpson, G. G. 1965. The Geography of Evolution. Chilton, Philadelphia.

Smith, F. D. M., R. M. May, R. Pellew, T. H. Johnson, and K. R. Walter. 1993. How much do we know about the current extinction rate? Trends Ecol. Evol. 8: 375–378.

Sneath, P. H. A. 1957. The application of computers to taxonomy. J. Gen. Microbiol. 17: 201–226.

_____ and R. R. Sokal. 1973. Numerical Taxonomy. W. H. Freeman, San Francisco.

Sokal, R. R., J. H. Camin, F. J. Rohlf, and P. H. A. Sneath. 1965. Numerical taxonomy: some points of view. Syst. Zool. 14: 237–243.

_____ and P. H. A. Sneath. 1963. Principles of Numerical Taxonomy. W. H. Freeman, San Francisco.

Soltis, P. S., D. E. Soltis, S. J. Novak, J. L. Schult, and R. K. Kuzoff. 1995. Fossil DNA: its potential for biosystematics. Pp. 1–13 *in* P. C. Hoch and A. G. Stephenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard. 53.

Stace, C. A. 1989. Plant Taxonomy and Biosystematics, 2nd ed. Edward Arnold, London.

Stork, N. E. 1988. Insect diversity: facts, fiction, and speculation. Biol. J. Linn. Soc. 35: 321–337.

Swain, T. (ed.) 1963. Chemical Plant Taxonomy. Academic Press, London.

Sytsma, K. J. and L. D. Gottlieb. 1986. Chloroplast DNA evidence for the origin of the genus *Heterogaura* from a species of Clarkia (Onagraceae). Proc. Natl. Acad. Sci. U.S.A. 83: 5554–5557.

_____ and W. J. Hahn. 1994. Molecular systematics: 1991–1993. Progr. Bot. 55: 307–333.

_____ and B. A. Schaal. 1985. Phylogenetics of the *Lisianthius skinneri* (Gentianaceae) species complex in Panama utilizing DNA restriction fragment analysis. Evolution 39: 594–608.

_____ and J. F. Smith. 1992 Molecular systematics of Onagraceae: examples from *Clarkia* and *Fuchsia*. Pp. 295–323 *in* P. S. Soltis, D. E. Soltis, and J. J. Doyle (eds.), Molecular Systematics of Plants. Chapman and Hall, New York.

_____, _____, and L. D. Gottlieb. 1990. Phylogenetics in *Clarkia* (Onagraceae): restriction site mapping of chloroplast DNA. Syst. Bot. 15: 280–295.

_____, _____, and P. C. Hoch. 1991. A chloroplast DNA analysis of tribal and generic relationships within Onagraceae. Amer. J. Bot. 78: 222. [Abstract.]

Tobe, H. and P. H. Raven. 1983. An embryological analysis of Myrtales: its definition and characteristics. Ann. Missouri Bot. Gard. 70: 71–94.

Vickery, R. K. 1984. Biosystematics 1983. Pp. 1–24 *in* W. F. Grant (ed.), Plant Biosystematics. Academic Press, New York.

Whittemore, A. T. and B. A. Schaal. 1991. Interspecific gene flow in sympatric oaks. Proc. Natl. Acad. Sci. U.S.A. 88: 2540–2544.

Wilson, E. O. (ed.) 1988. Biodiversity. National Academy Press, Washington.

_____. 1989. The coming pluralization of biology and the stewardship of systematics. Bioscience 39: 242–245.

FOSSIL DNA: ITS POTENTIAL FOR BIOSYSTEMATICS[1]

Pamela S. Soltis[2], Douglas E. Soltis[2], Stephen J. Novak[3],
Joanna L. Schultz[2], and Robert K. Kuzoff[2]

ABSTRACT. Studies of plant phylogeny, biogeography, and molecular evolution can all benefit tremendously from molecular data obtained from fossil specimens. Herein we review briefly the ages and taxonomic distribution of those ancient DNAs examined to date and emphasize the DNA sequence data from Miocene plant fossils from Clarkia, Idaho. The potential of fossil DNAs for resolving phylogenetic relationships, exploring biogeographic patterns, and documenting rates and patterns of molecular evolution is explored. The primary limitations of molecular paleobotany are also discussed. Problems of DNA degradation and extraction, amplification of target DNA sequences via the polymerase chain reaction, contamination, and verification must all be overcome before analysis of ancient DNA represents anything more than a biological curiosity.

The field of biosystematics focuses on inferring genealogical relationships and interpreting historical processes that have given rise to extant patterns of organismal diversity. Because our understanding of evolutionary patterns is based on living organisms, it is undoubtedly marred by extinction events that are both recognized and unknown. Inferences of both evolutionary patterns and processes have benefited tremendously from the application of molecular techniques to evolutionary biology. Even greater confidence in hypotheses of phylogenetic relationships and past evolutionary processes could be gained through analysis of DNA obtained from fossils. In addition, studies of biogeography and molecular evolution would also benefit greatly from the analysis of ancient DNAs.

The past decade has witnessed the isolation and analysis of ancient DNAs representing a diversity of ages, organisms, and modes of preservation (reviewed in Soltis and Soltis, 1993). For example, DNAs have been isolated from materials ranging in age from a few hundred years (e.g., the extinct quagga [Higuchi et al., 1984] and marsupial wolf [Thomas et al., 1989]) to several million years. Miocene leaves of *Magnolia* (Golenberg et al., 1990) and *Taxodium* (Soltis et al., 1992) have yielded chloroplast DNA (cpDNA) sufficiently well preserved to permit sequence analysis of most of *rbc*L, the gene encoding ribulose 1,5-bisphosphate carboxylase/oxygenase. Smaller DNA fragments have been analyzed from Oligo-Miocene termites (DeSalle et al., 1992), stingless bees (Cano et al., 1992), and legumes (Poinar et al., 1993) and from Cretaceous weevils (Cano et al., 1993). Because most of the DNA extracted from preserved tissues is highly degraded (see also Rogers and Bendich, 1985; Pääbo, 1985a, 1985b, 1990; Pääbo et al., 1988; Lawlor et al., 1991), analysis of the DNA relies on the polymerase chain reaction (PCR) to amplify target sequences.

The Miocene plant DNAs have yielded the longest gene sequences to date, 759 base pairs (bp) from *Magnolia latahensis* (Golenberg et al., 1990) and 1320 bp from *Taxodium* (Soltis et al., 1992), suggesting perhaps that the chloroplast membrane surrounding the cpDNA and/or the mode of preservation of these leaves has limited the extent of DNA degradation. In contrast, nuclear and mitochondrial DNAs from other sources appear more degraded. Amplified products of nuclear 18S and mitochondrial 12S genes are typically only a few hundred bp in length (e.g., DeSalle et al., 1992; Janczewski et al., 1992; Cano et al., 1992, 1993).

[1]This work was supported by NSF Grant BSR-9201749. We thank Mike Clegg, Ed Golenberg, and Jack Smiley for valuable discussions and Francis and Vicki Kienbaum and the Tertiary Research Center of the University of Idaho for access to the Clarkia fossil site.
[2]Department of Botany, Washington State University, Pullman, Washington 99164-4238, U.S.A.
[3]Department of Biology, Boise State University, Boise, Idaho 83725, U.S.A.

MIOCENE DNAs

The Clarkia Fossil Site: Early to mid-Miocene deposits in the St. Maries River Valley near Clarkia, Idaho, U.S.A., contain spectacular fossil beds rich in plant megafossils. These fossil beds rest on a basement of mica schists of the Wallace Group of the Belt Supergroup (Hietanen, 1963; Clark, 1963) and are covered by a mantle of one meter of loess soil of Quaternary age. The Proto-St. Maries River drainage was formed during the Paleogene, and the river was dammed by a lava flow in the early Neogene, forming the Miocene Clarkia Lake (Smiley and Rember, 1981, 1985). Rapid sedimentation of the lake, in some areas taking perhaps only a few decades (Smiley and Rember, 1985), trapped abundant plant material that has been preserved as unoxidized compression fossils. The lacustrine sediments and their fossils have remained water-saturated since their deposition, preserving the cellular integrity, organelles, organic chemistry, and original pigmentation of the plant material (Figure 1). In fact, some leaf specimens can be removed intact from the rock surfaces. The rapid, anaerobic preservation of undamaged leaves and the subsequent water-saturation of the deposits have combined to maintain the cellular and subcellular structures of the leaves (e.g., Niklas et al., 1985). Other fossil sites of similar age and composition in the Columbia Plateau of the Pacific Northwest cannot boast this mode of preservation. Higher precipitation in the Northern Rocky Mountains (660 mm annual precipitation) has maintained the saturation of the deposits, contributing to the outstanding preservation of these fossils (Smiley et al., 1975), whereas similar fossiliferous deposits in the more xeric regions (<260 mm) of the Columbia Plateau have been oxidized by periodic water flows that have leached away organic material, leaving only fossil imprints (Smiley and Rember, 1985).

Figure 1. Fossil specimen of *Liquidambar* from the Clarkia site.

The precise age of the fossils from Clarkia Lake is still uncertain. Potassium-argon dates obtained from the basalts of the area range from 15.4 million years before present to 22 million years before present (reviewed in Smiley and Rember, 1985). The paleoclimatic and floristic data suggest an early Miocene age of perhaps 20 million years before present, to as recently as 17 million years before present (Smiley and Rember, 1985).

These fossil beds have provided material for morphological, anatomical, ultrastructural, and biochemical analyses of Miocene plants (reviewed in Smiley, 1985). In addition, fossil specimens from Clarkia have provided some of the most ancient, and perhaps the best preserved, sources of fossil DNA to date (Golenberg et al., 1990; Soltis et al., 1992).

Magnolia latahensis. A major breakthrough in the study of ancient DNAs came in 1990 with the analysis of a chloroplast DNA (cpDNA) sequence from a Miocene *Magnolia* (Golenberg et al., 1990). Golenberg et al. (1990) isolated DNA from the now-extinct *M. latahensis* obtained from the Clarkia site. They used the PCR to amplify an 820-bp portion of *rbc*L and obtained a 759-bp sequence from this amplified product. They also compared the fossil *Magnolia* sequence with *rbc*L sequences of other Magnoliaceae. This study demonstrated that the age of DNA that can be successfully isolated and sequenced is considerably greater than previous attempts indicated.

The validity of the work of Golenberg et al. (1990) was questioned, primarily on theoretical grounds (Pääbo and Wilson, 1991) because *in vitro* studies of DNA degradation (Lindahl and Andersson, 1972; Lindahl and Nyberg, 1972) suggested to Pääbo and Wilson (1991) that DNA should be completely degraded after only four million years. In addition, Pääbo and Wilson (1991) and Sidow et al. (1991) also questioned the potential value of the Clarkia fossils for molecular study by suggesting that even if DNA can *occasionally* be isolated from a Miocene fossil, the repeatability of such analyses is likely very low.

Taxodium. We initiated an investigation to test the repeatability of Miocene DNA analysis and sequenced *rbc*L from a fossil specimen of *Taxodium* (bald cypress; Soltis et al., 1992). To isolate fossil DNAs, fossil-bearing rocks were removed from the hillside and cracked open to expose the fossils. The fossils were identified and photographed, and then the leaf material was scraped from the rocks into a mortar. The fossil leaf material was ground on dry ice, and the DNA was isolated following a basic CTAB extraction procedure (modified from Doyle and Doyle, 1987). DNA pellets were resuspended in 25 µl TE.

We amplified a 1380-bp portion of *rbc*L (a gene typically approximately 1428 bp in length) from the fossil *Taxodium* specimen. The 5' PCR primer was composed of the first 30 bases of *rbc*L of *Zea mays*; the 3' primer consisted of bp 1351-1380 of maize *rbc*L on the reverse strand (primer sequences given in Soltis et al., 1992). PCR conditions consisted of 30 cycles of 1.5 min at 94°C, 2 min at 42°C, and 3 min at 72°C, followed by a final 15-min extension at 72°C and a holding temperature of 20°C. We then produced single-stranded (ss) DNAs for sequencing through asymmetric PCR using the dsDNA as a template and the two primers individually. The ssDNAs were sequenced using the dideoxy chain termination method (Sanger et al., 1977) and primers derived from maize (provided by G. Zurawski). This procedure resulted in 1320 bp of fossil *Taxodium* sequence. Each strand was sequenced two or three times to confirm the sequence. For comparison, we also initially amplified and sequenced *rbc*L from DNAs of two other members of the Taxodiaceae: extant *T. distichum* and *Metasequoia glyptostroboides*. Subsequent analyses included representatives of all genera of Taxodiaceae and several genera of Cupressaceae (Brunsfeld et al., 1994).

The fossil *rbc*L sequence differs from the extant *Taxodium* sequence by 11 base substitutions of 1320 bp examined, for a sequence divergence of 0.83%. All of the substitutions

were transitions and silent third-position substitutions. Molecular divergence between the fossil and extant *Taxodium* sequences has been relatively limited, compared with the amount of divergence between *rbc*L sequences of Miocene fossil *Magnolia latahensis* and extant *M. macrophylla*. Golenberg et al. (1990) detected 17 substitutions of only 759 positions examined (2.2% sequence divergence), almost three times what we observed for the Taxodiums. Of the 17 substitutions reported for the *Magnolia* species, 12 were transitions and five were transversions; all but four of the changes were silent third-position substitutions. *rbc*L sequence divergence between *M. latahensis* and several other species of *Magnolia* is also extensive (Qiu et al., 1993). The lesser divergence between the sequences from fossil and extant *Taxodium* relative to those of fossil and extant *Magnolia* species may reflect the apparent conspecific status of the *Taxodium* samples. Although the Miocene *Taxodium* from northern Idaho is not referred to as *T. distichum*, the fossil specimens are usually not recognized taxonomically as a distinct species either (C. J. Smiley, pers. comm.). In contrast, although *M. latahensis* is similar in its leaf morphology and gynoecia to several eastern North American species of *Magnolia*, including *M. macrophylla*, *M. grandiflora*, and *M. virginiana*, it is considered a species of the Columbia Plateau fossil floras.

The rate of sequence divergence between the *Taxodium* samples can be determined by dividing the number of substitutions by the time since divergence. Although the time since divergence of the two populations of these *Taxodium* samples is uncertain, it is at least the age of the Clarkia site (17–20 million years) and most likely longer. If the two Taxodiums actually shared a common ancestor 17–20 million years ago, this amounts to a minimum sequence divergence rate of 0.55–0.65 substitutions per million years along the length of the gene (1320 bp) or $4.2 \times 10^{-4} - 4.9 \times 10^{-4}$ substitutions per site per million years. Populations of *Taxodium* from northern Idaho and eastern North America have undoubtedly been genetically isolated even longer given the distance between these areas, suggesting an even slower rate of nucleotide substitution (see further discussion in Brunsfeld et al., 1994).

Initial phylogenetic analyses of the *rbc*L sequences of the *Taxodium* fossil, extant *T. distichum*, *M. glyptostroboides*, *Pseudotsuga menziesii* (Pinaceae), and the liverwort *Marchantia polymorpha* (as the outgroup) demonstrated the similarity of the two *Taxodium* sequences and their similarity to the sequence of the confamilial *M. glyptostroboides* (Soltis et al., 1992). All nodes were recognized in all trees produced in 100 bootstrap replicates using Wagner parsimony, and the identical tree topology was obtained with the Fitch-Margoliash and maximum likelihood procedures. We have recently included the fossil *Taxodium* sequence in a larger analysis of Taxodiaceae/Cupressaceae (Brunsfeld et al., 1994), with *Araucaria* used as the outgroup. Although the relative positions of some lineages of Taxodiaceae are uncertain, the fossil and extant *Taxodium* sequences consistently appear as sister taxa (Figure 2), with 100% bootstrap support and a decay index of >5 steps.

The successful amplification and sequencing of the majority of the *rbc*L gene from a Miocene *Taxodium* confirms the possibility of analyzing DNA dating from 17–20 million years ago and also demonstrates the repeatability of this type of analysis. Repeated success with amber-preserved material of Oligo-Miocene age (DeSalle et al., 1992; Cano et al., 1992) also indicates that rates of DNA degradation may be slowed by certain modes of preservation. Because the Clarkia flora is represented by three ecological communities (Smiley and Rember, 1980)—the swamp association, to which *Taxodium* belongs, the floodplain-slope association, and the drier-slope association—this fossil flora is highly diverse taxonomically and suggests numerous opportunities for research on a wide variety of gymnosperms and angiosperms. The Miocene fossils from northern Idaho may hold great potential for studies of molecular evolution, biogeography, and phylogenetic relationships of both plants and animals.

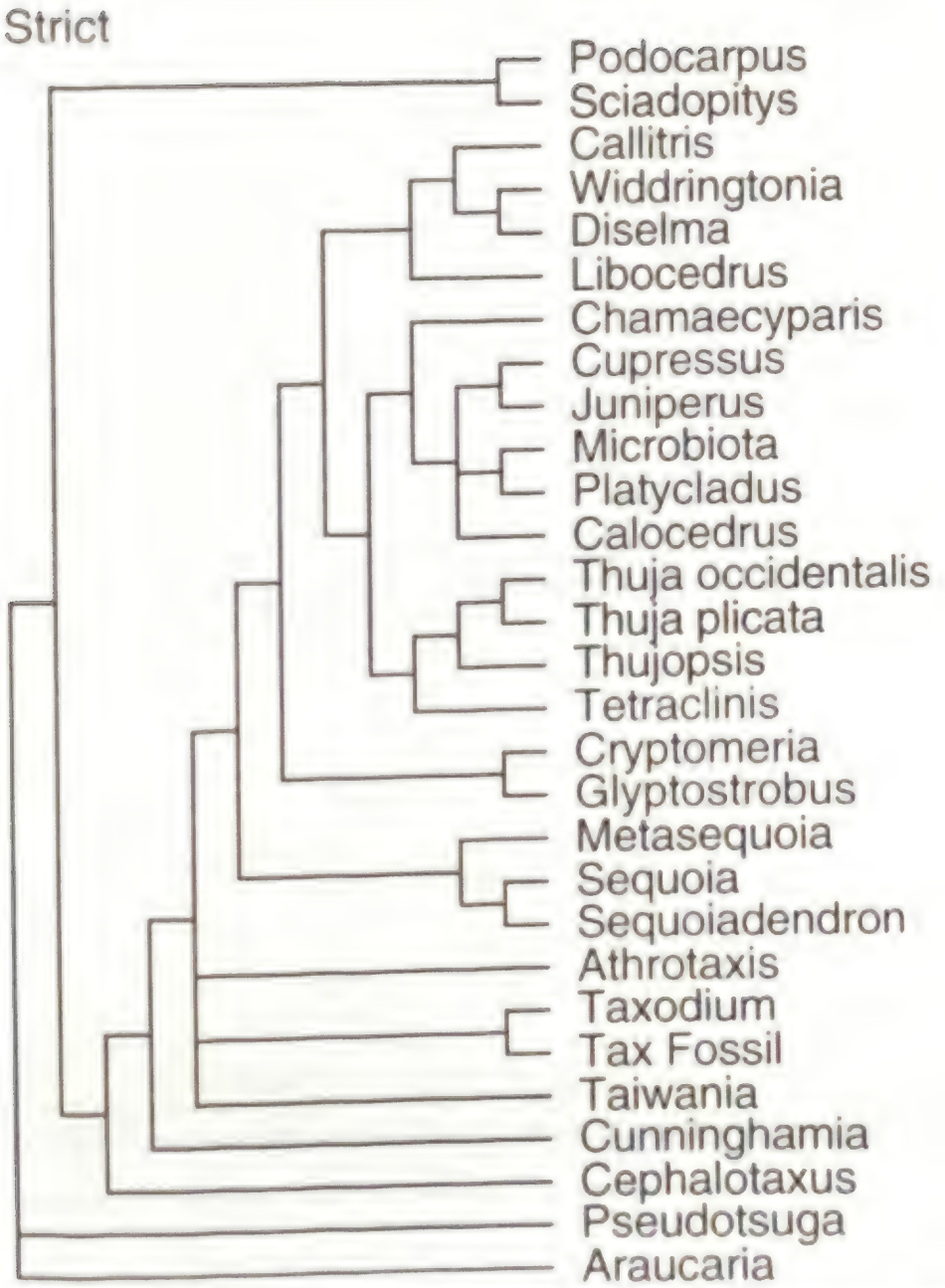


Figure 2 Strict consensus tree showing relationships among genera of Taxodiaceae and representative Cupressaceae based on *rbcL* sequences. Note sister status of fossil and extant *Taxodium* sequences (from Brunsfeld et al. 1994).

POTENTIAL OF FOSSIL DNA: EXAMPLES FROM THE CLARKIA FLORA

Phylogenetic Relationships. Using this same general approach of sequence analysis and verification, a variety of systematic and evolutionary questions can be addressed. Fossil DNA sequences can be used to evaluate phylogenetic relationships of fossil taxa to extant ones. At the same time, sequences from fossil specimens can be used to "identify" the fossils themselves. An example of how these two objectives can be addressed simultaneously involves the extinct genus *Nordenskioldia*. This genus dates from the late Cretaceous and is particularly common in Paleocene deposits from the Northern Hemisphere, including the Pacific Northwest of the U.S.A. (Crane et al., 1991) and the Miocene deposits at Clarkia (Manchester et al., 1991). The name *Nordenskioldia* is assigned to the infructescences, which clearly belong to Trochodendrales (Crane et al., 1991). Crane et al. (1991) proposed that *Nordenskioldia* is most closely related to *Trochodendron* and that relationships in Trochodendraceae follow the scheme in Figure 3. Although the fruits are distinctive, the leaves now recognized as being associated with these fruits (Kryshtofovich, 1956; Crane et al., 1991) have been classified in such diverse genera as *Populus* of Salicaceae (Knowlton, 1926) and *Cocculus* of Menispermaceae (LaMotte, 1952). Most recently, the leaves of a *"Nordenskioldia"* plant have been recognized as the extinct *Zizyphoides* (Crane et al., 1991). DNA sequences could be used to test several hypotheses regarding *Nordenskioldia*: (1) DNA isolated from both fruits and leaves could be compared to determine whether or not the leaves truly are part of the *Nordenskioldia* plant. In this way, fossil sequences could help to identify the fossil specimen. (2) Comparing sequences of *Zizyphoides, Nordenskioldia, Trochodendron, Tetracentron, Cercidiphyllum,* and other Trochodendrales could test the relationships among the genera of this order. Similar examples in a diversity of plant groups await molecular study.

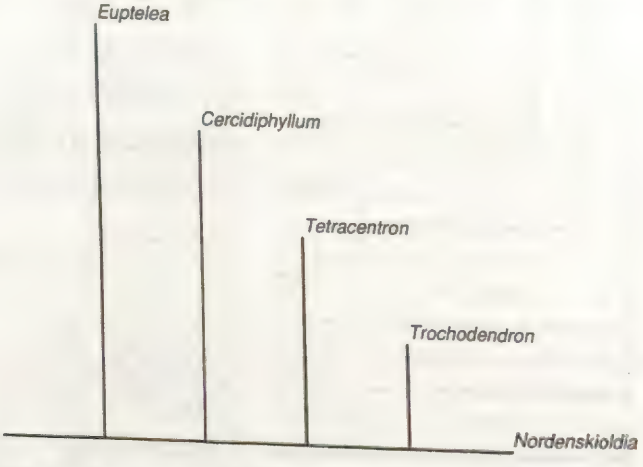


Figure 3. Proposed relationships among genera of Trochodendrales (redrawn from Crane et al., 1991).

Biogeography. Fossil DNA sequences can also be used to examine biogeographic patterns. The Miocene deposits at Clarkia reveal a warm temperate forest similar in structure and composition to those currently found in eastern Asia and eastern North America. This disjunction pattern, first discussed by Linnaeus (in Halenius, 1750) and developed more fully by Gray (1859) and others, probably arose through range restrictions of more widespread floras during the late Tertiary (Chaney, 1947; Axelrod, 1960; Thomas and Spicer, 1987). The Taxodiaceae exhibit a common floristic disjunction, with some genera located in eastern Asia,

some from western North America, and some from eastern North America. *Taxodium* currently occurs in eastern North America, *Sequoia* is restricted to western North America, and *Metasequoia* and *Cunninghamia* are found in eastern Asia. In addition, these four genera are present in the Miocene deposits at Clarkia, despite their current highly disjunct distributions. Thus, analysis of fossil materials of these genera, in addition to extant Taxodiaceae of these and other genera, could provide some important insight into the past distributions, relationships, and divergence of these genera, and contribute to ongoing phylogenetic studies of Taxodiaceae (Brunsfeld et al., 1994; Figure 2) and Cupressaceae (e.g., Gadek and Quinn, 1993).

Similar studies could also be conducted on other groups, such as *Liriodendron* and *Liquidambar*, that also exhibit major floristic disjunctions. For example, genetic divergence between eastern Asian and eastern North American species of *Liriodendron* has been estimated using both allozymes and cpDNA restriction site data (Parks and Wendel, 1990). cpDNA divergence between the two extant species, *L. tulipifera* of eastern North America and *L. chinense* of eastern Asia, was estimated to be 1.24%. Given that both molecular data sets and paleobotanical evidence suggest a divergence time of 10–16 million years before present, this level of divergence is consistent with that observed between extant *T. distichum* and the Miocene fossil *Taxodium*. A clearer picture of both patterns and rates of divergence in *Liriodendron* could be obtained by incorporating molecular data from Miocene fossils from Clarkia.

Molecular Evolution. Fossil DNA sequences also provide a unique perspective for the study of molecular evolution. The molecular clock hypothesis, first proposed by Zuckerkandl and Pauling (1965), suggests that nucleotide substitutions occur in a regular, clock-like manner. Thus, if this were true, one could estimate the time since the divergence of two lineages by recording the number of nucleotide differences between them. Although this general method has been used on numerous occasions, often in conjunction with fossil dates, to estimate the time since two or more lineages diverged (e.g., Sarich and Wilson, 1967; Beverley and Wilson, 1985; Bowen and Avise, 1989; Wolfe et al., 1989; Clegg, 1990), evidence has been presented that questions the validity of the molecular clock. Rates of nucleotide substitution vary dramatically among lineages (e.g., Britten, 1986; Wu and Li, 1985; Wilson et al., 1990) and even through time in a single lineage (e.g., Melnick, 1990). Therefore, at best, the molecular clock ticks at different rates in different lineages and, at worst, moves irregularly, at least occasionally precluding its use in comparing rates of sequence divergence across lineages and in estimating divergence times. Furthermore, the view of the molecular clock also varies with the gene under analysis, because different genes (and different genomes) evolve at different rates (e.g., Brown et al., 1979; Wolfe et al., 1987). Thus, estimates of divergence times based strictly on the number of nucleotide substitutions observed could vary greatly from gene to gene.

However, fossil DNA, such as that retrievable from the Miocene fossils at Clarkia, holds great promise for studies of molecular evolution. By anchoring the time of organismal divergence in the fossil record, it becomes possible to calibrate rates of sequence divergence over a known time-frame, even for genes that evolve at different rates. This is a much more reliable way of calibrating the molecular clock than by trying to estimate divergence times between related extant taxa through comparisons of sequence divergence. In addition, by evaluating rates of divergence between fossil sequences and those of extant species in a number of different groups, one can compare rates of sequence divergence among lineages. Through these comparisons, it may be possible to understand the factors that control evolutionary rates and test further the usefulness of the concept of the molecular clock over long and short time periods.

LIMITATIONS OF FOSSIL DNA

To this point we have emphasized the *potential* of fossil specimens for molecular and evolutionary analysis. However, this type of work also faces many limitations, including problems with DNA extraction, confirming that the DNA extracted is truly from the fossil specimen and not from microorganisms associated with the fossil specimen, and DNA amplification.

DNA Extraction. The first obstacle is extracting DNA from the fossil material, and success of extraction varies with the taxon and the quality of preservation. Conifer fossils from the Clarkia site required additional dry ice during the initial grinding to reduce the resinous nature of the samples. No other samples required special treatment to obtain extractions of standard appearance. A list of all taxa from which DNA extractions were attempted, and whether or not these extractions yielded DNA, is given in Table 1. Samples of DNA extracted from fossil specimens using the CTAB procedure described for *Taxodium* are shown in Figure 4 in an agarose gel stained with ethidium bromide and photographed under UV light. DNA is clearly visible in a number of these lanes. Overall, approximately 20% of all samples examined have yielded visible DNA. Similar success rates of 10–15% have been reported by other investigators of Miocene DNAs (Golenberg et al., 1990, pers. comm.). Perhaps extraction procedures that use Chelex (e.g., Cano et al., 1992) or do not require grinding of the tissue (Ghingan, 1992) may improve the yield of DNA from fossil specimens.

Figure 4. DNA extracts from fossil specimens electrophoresed through an agarose gel, stained with ethidium bromide, and photographed under UV light. Note presence of high molecular-weight DNA in lanes designated by arrows.

Plant vs. Bacterial DNA. The question arises, however, as to whether or not the DNA visible is derived from the fossil specimens. Sidow et al. (1991) recently demonstrated that many of the DNA samples they obtained from Clarkia fossils in fact derive not from fossilized plants but from bacteria. Sidow et al. (1991) obtained relatively high molecular-weight (>10 kb) DNA from two of seven samples, but were unable to amplify the plant gene *rbc*L from these extracts. However, they successfully amplified a 370-bp portion of the bacterial 16S ribosomal RNA gene from samples with and without visible DNA. Furthermore, this PCR-derived bacterial DNA sequence hybridized to the high molecular-weight fossil DNA extracts from which it was derived and to *E. coli* DNA, further indicating that the visible high molecular-weight DNA was actually bacterial in origin. Whether these bacteria are extant, having penetrated the fossiliferous strata, or Miocene in origin could not be determined by their study. That bacterial DNA is present in the Miocene plant fossil DNA extracts from Clarkia need not necessarily present a problem for analysis of fossil plant DNA sequences. Amplification of cpDNA sequences will most likely involve primers specific to cpDNA, precluding inadvertent amplification of bacterial or fungal sequences. Furthermore, Sidow et al. (1991) recommend that further attempts to amplify plant sequences from Clarkia fossils should not be restricted to those extracts containing visible high molecular-weight DNA.

Table 1. Number of Miocene specimens from which DNA extractions were attempted, and from which visible DNA was successfully extracted.

Genus (Family)	Number of Samples	Number with Visible DNA
Conifers		
Pinus (Pinaceae)	1	1
Metasequoia (Taxodiaceae)	4	1
Taxodium (Taxodiaceae)	14	5
Angiosperms		
Betula (Betulaceae)	4	1
Castanea or *Pseudofagus* (Fagaceae)	1	0
Castanopsis (?) (Fagaceae)	1	0
"*Cocculus*" (Menispermaceae)	11	2
Diospyros (Ebenaceae)	1	0
Fagus (Fagaceae)	5	0
Halesia (Styracaceae)	1	0
Hydrangea (Hydrangeaceae)	2	1
Legumes (unknown) (Fabaceae)	3	0
Lindera (Lauraceae)	4	1
Liquidambar (Hamamelidaceae)	9	3
Liriodendron (Magnoliaceae)	3	2
Lithocarpus (Fagaceae)	4	0
*Magnolia** (Magnoliaceae)	20	1
Malva (?) (Malvaceae)	2	0
Nyssa (Nyssaceae)	3	0
Paulownia (Scrophulariaceae)	1	1
Persea (Lauraceae)	7	2
Platanus (Platanaceae)	15	5
Pseudofagus (Fagaceae)	20	6
Quercus (Fagaceae)	17	1
Rosaceae, unknown	1	0
Smilax (Liliaceae)	2	1
Symplocus (Symplocaceae)	1	1
Symplocus or *Halesia*	2	1
Vitis (Vitaceae)	1	0
Zelkova (Ulmaceae)	1	0
Zenia (Leguminosae)	6	0

*Includes one sample that could not definitively be distinguished from *Persea* (Lauraceae) and a second sample that could not be distinguished from *Asimina* (Annonaceae).

Amplification of Target Sequences. Amplified products may be difficult to obtain even from DNA that is truly extracted from the fossil itself. The clay matrix surrounding the fossil material may be included in the DNA solution or may even be bound to the DNA. These substances may prevent the successful action of the heat-stable polymerase to amplify a target DNA sequence. The addition of bovine serum albumin to the DNA extracts may remove some of these external materials and allow the PCR to proceed. Excess polymerase may also enhance PCR reactions when inhibiting molecules are present (e.g., Pääbo et al., 1988). In addition, inhibiting compounds may be physically removed from the DNA by agarose gel electrophoresis. More elaborate methods of cleaning the DNA may also be necessary.

Because the fossil DNA samples may be largely degraded, there may be relatively little high molecular-weight DNA to serve as template in PCR reactions. Thus, primer combi-

nations designed to amplify a large portion of DNA, such as the Zl-1351R combination to amplify a 1.38-kb fragment of *rbc*L, may not be successful because insufficient template is present. To address this problem, we have also used primer combinations representing smaller portions of *rbc*L in PCR reactions (Figure 5). Recent studies of Oligo-Miocene (DeSalle et al., 1992; Cano et al., 1992) and Cretaceous (Cano et al., 1993) DNAs have amplified and sequenced small fragments (<300 bp) of genes.

An additional problem associated with amplification of fossil DNA sequences (or any template present in low concentration) is the inadvertent amplification of sequences from DNA other than the target. The probability of amplifying bacterial or fungal DNA sequences rather than target fossil plant sequences can be reduced by amplification of plant-specific genes from the chloroplast, mitochondrial, or nuclear genomes. However, contamination of solutions used in PCR reactions from other plant DNA sources can interfere with the successful amplification of the target sequence. Because even a small amount of contaminant DNA can swamp the amplification of the target, extreme care must be taken to avoid contamination, and appropriate control PCR reactions should be performed in parallel with target amplifications (reviewed by Pääbo, 1990).

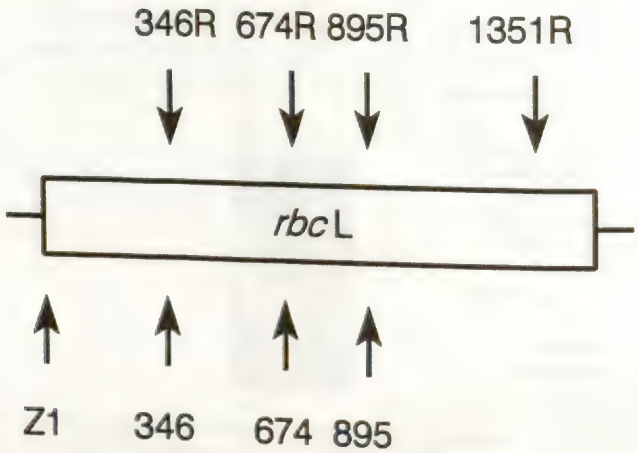


Figure 5. Positions of primers used in amplification of *rbc*L from fossil DNAs. Primer sequences are based on the *rbc*L sequence of *Zea mays*.

FEASIBILITY OF FOSSIL DNA STUDIES

We have conducted a feasibility study of the extraction and analysis of Miocene DNA from the Clarkia site. Of the approximately 166 samples analyzed to date, 36 (22%) have high molecular-weight DNA [but see section above on *Plant vs. Bacterial DNA* and Sidow et al. (1991)]. However, all PCR amplifications of *rbc*L (or portions of *rbc*L) other than that of the *Taxodium* fossil specimen (Soltis et al., 1992) have failed. Thus, the success rate for obtaining fossil sequences is very low. Other labs actively engaged in DNA analysis of Clarkia fossils have met with similar frustrations (M. Clegg et al., pers. comm.; E. Golenberg, pers. comm.; H. Wichmann et al., pers. comm.). Therefore, the very real limitations associated with isolation and analysis of Miocene DNAs should be carefully considered before undertaking any broad-scale study of fossil specimens. Without improvements in methods of DNA extraction and amplification from fossils, it will be difficult to analyze large numbers of fossil DNA sequences. However, the rewards of retrieving Miocene and other ancient DNA sequences are tremendous and well worth continued effort.

LITERATURE CITED

Axelrod, D. I. 1960. The evolution of flowering plants. Pp. 227–305 *in* S. Tax (ed.), Evolution after Darwin, Vol. 1. University of Chicago Press, Chicago.

Beverley, S. M. and A. C. Wilson. 1985. Ancient origin for Hawaiian Drosophilinae inferred from protein comparisons. Proc. Natl. Acad. Sci. U.S.A. 82: 4753–4757.

Bowen, B. W. and J. C. Avise. 1989. Odyssey of the green sea turtle: Ascension Island revisited. Proc. Natl. Acad. Sci. U.S.A. 86: 573–576.

Britten, R. J. 1986. Rates of DNA sequence evolution differ between taxonomic groups. Science 231: 1393–1398.

Brown, W. M., M. George, Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. 76: 1967–1971.

Brunsfeld, S. J., P. S. Soltis, D. E. Soltis, P. A. Gadek, C. J. Quinn, D. D. Strenge, and T. A. Ranker. 1994. Phylogenetic relationships among the genera of Taxodiaceae and Cupressaceae: evidence from *rbc*L sequences. Syst. Bot. 19: 253–262.

Cano, R. J., H. N. Poinar, D. W. Roubik, and G. O. Poinar, Jr. 1992. Enzymatic amplification and nucleotide sequencing of portions of the 18S rRNA gene of the bee *Proplebeia dominicana* (Apidae: Hymenoptera) isolated from 25–40 million year-old Dominican amber. Med. Sci. Res. 20: 619–622.

_____, _____, N. J. Pieniazek, A. Acra, and G. O. Poinar, Jr. 1993. Amplification and sequencing of DNA from a 120–135-million-year-old weevil. Nature 363: 536–538.

Chaney, R. W. 1947. Tertiary centers and migration routes. Ecol. Monogr. 17: 139–148.

Clark, A. L. 1963. Geology of the Clarkia area, Idaho. M.S. Thesis, University of Idaho, Moscow.

Clegg, M. T. 1990. Dating the monocot-dicot divergence. Trends Ecol. Evol. 5: 1–2.

Crane, P. R., S. R. Manchester, and D. L. Dilcher. 1991. Reproductive and vegetative structure of *Nordenskioldia* (Trochodendraceae), a vesselless dicotyledon from the early Tertiary of the Northern Hemisphere. Amer. J. Bot. 78: 1311–1334.

DeSalle, R., J. Gatesy, W. Wheeler, and D. Grimaldi. 1992. DNA sequences from a fossil termite in Oligo-Miocene amber and their phylogenetic implications. Science 257: 1933–1936.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Gadek, P. A. and C. J. Quinn. 1993. A preliminary analysis of relationships within the Cupressaceae *sensu stricto* based on *rbc*L sequences. Ann. Missouri Bot. Gard. 80: 581–586.

Ghingan, N. K. 1992. A novel technology for DNA isolation. Meth. Molec. Cell Biol. 3: 15–22.

Golenberg, E. M., D. E. Giannasi, M. T. Clegg, C. J. Smiley, M. Durbin, D. Henderson, and G. Zurawski. 1990. Chloroplast DNA sequence from a Miocene *Magnolia* species. Nature 344: 656–658.

Gray, A. 1859. Diagnostic characters of new species of phanerogamous plants collected in Japan by Charles Wright, Botanist of the U. S. North Pacific Exploring Expedition. (Published by request of Captain James Rodgers, Commander of the Expedition.) With observations upon the relations of the Japanese flora to that of North America, and of other parts of the North Temperate Zone. Mem. Amer. Acad. Arts 6: 377–452. (Reprinted in part in Stuckey, 1978.)

Halenius, J. 1750. Plantae Rariores Camschatcenses. Thesis. University of Uppsala, Uppsala, Sweden.

Hietanen, A. 1963. Metamorphism of the Belt Series in the Elk River-Clarkia area of Idaho. U. S. Geol. Surv. Profess. Pap. 344-C.

Higuchi, R., B. Bowman, M. Friedberger, O. A. Ryder, and A. C. Wilson. 1984. DNA sequences from the quagga, an extinct member of the horse family. Nature 312: 282–284.

Janczewski, D. N., N. Yuhki, D. A. Gilbert, G. T. Jefferson, and S. J. O'Brien. 1992. Molecular phylogenetic inference from saber-toothed cat fossils of Rancho LaBrea. Proc. Natl. Acad. Sci. U.S.A. 89: 9769–9773.

Knowlton, F. H. 1926. Flora of the Latah Formation of Spokane, Washington, and Coeur d'Alene, Idaho. U. S. Geol. Surv. Profess. Pap. 140-A: 17–82.

Kryshtofovich, A. N. 1956. Oligocene flora of Mount Ashutus in Kazahkstan. Paleobotanica Fasciculus 1. Institutum Botanicum nomine V. L. Komarovii Academiae Scientarium URSS. ACTA Series 8, Moscow.

LaMotte, R. S. 1952. Catalogue of the Cenozoic plants of North America through 1950. Geol. Soc. Amer. Mem. 51: 1–381.

Lawlor, D. A., C. D. Dickel, W. W. Hauswirth, and P. Parham. 1991. Ancient *HLA* genes from 7,500-year-old archaeological remains. Nature 349: 785–787.

Lindahl, T. and A. Andersson. 1972. Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. Biochemistry 11: 3618–3623.

_____ and B. Nyberg. 1972. Rate of depurination of native deoxyribonucleic acid. Biochemistry 11: 3610–3618.

Manchester, S. R., P. R. Crane, and D. L. Dilcher. 1991. *Nordenskioldia* and *Trochodendron* (Trochodendraceae) from the Miocene of northwestern North America. Bot. Gaz. 152: 357–368.

Melnick, D. 1990. Molecules, evolution, and time. Trends Ecol. Evol. 5: 172–173.

Niklas, K. J., R. M. Brown, Jr., and R. Santos. 1985. Ultrastructural states of preservation in Clarkia angiosperm leaf tissues: implications on modes of fossilization. Pp. 143–160 *in* C. J. Smiley (ed.), Late Cenozoic History of the Pacific Northwest. Pacific Division of the American Association for the Advancement of Science, San Francisco.

Pääbo, S. 1985a. Molecular cloning of ancient Egyptian mummy DNA. Nature 314: 644–645.

_____. 1985b. Preservation of DNA in ancient Egyptian mummies. J. Archaeol. Sci. 12: 411–417.

_____. 1990. Amplifying ancient DNA. Pp. 159–166 *in* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego.

_____ and A. C. Wilson. 1991. Miocene DNA sequences—A dream come true? Curr. Biol. 1: 45–46.

_____, J. A. Gifford, and A. C. Wilson. 1988. Mitochondrial DNA sequences from a 7000-year-old-brain. Nucl. Acids Res. 16: 9775–9787.

Parks, C. A. and J. F. Wendel. 1990. Molecular divergence between Asian and North American species of *Liriodendron* (Magnoliaceae) with implications for interpretation of fossil floras. Amer. J. Bot. 77: 1243–1256.

Poinar, H. N., R. J. Cano, and G. O. Poinar, Jr. 1993. DNA from an extinct plant. Nature 363: 677.

Qiu, Y.-L., M. W. Chase, and C. R. Parks. 1993. A phylogenetic reappraisal of the fossil *Magnolia rbc*L sequence. Amer. J. Bot. 80: 172–173.

Rogers, S. O. and A. J. Bendich. 1985. Extraction of DNA from milligram amounts of fresh, herbarium, and mummified plant tissues. Pl. Molec. Biol. 5: 69–76.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. 74: 5463–5467.

Sarich, V. and A. C. Wilson. 1967. Immunological time scale for hominid evolution. Science 158: 1200–1203.

Sidow, A., A. C. Wilson, and S. Pääbo. 1991. Bacterial DNA in Clarkia fossils. Philos. Trans., Ser. B 333: 429–433.

Smiley, C. J. (ed.). 1985. Late Cenozoic History of the Pacific Northwest. Pacific Division of the American Association for the Advancement of Science, San Francisco.

_____ and W. C. Rember. 1981. Paleoecology of the Miocene Clarkia Lake (Northern Idaho) and its environs. Pp. 551–590 *in* J. Gray, A. J. Boucot, and W. B. N. Berry (eds.), Communities of the Past. Dowden, Hutchinson, and Ross, Stroudsburg, PA.

_____ and _____ . 1985. Physical settings of the Miocene Clarkia fossil beds, northern Idaho. Pp. 11–31 *in* C. J. Smiley (ed.), Late Cenozoic History of the Pacific Northwest. Pacific Division of the American Association for the Advancement of Science, San Francisco.

_____, J. Gray, and L. M. Huggins. 1975. Preservation of Miocene fossils in unoxidized lake deposits, Clarkia, Idaho. J. Paleontol. 49: 833–844.

Soltis, P. S. and D. E. Soltis. 1993. Ancient DNA: prospects and limitations. New Zealand J. Bot. 31: 203–209.

_____, _____, and C. J. Smiley. 1992. An *rbc*L sequence from a Miocene *Taxodium* (bald cypress). Proc. Natl. Acad. Sci. U.S.A. 89: 449–451.

Stuckey, R. L. 1978. Essays on North American Plant Geography from the Nineteenth Century. Arno Press, New York.

Thomas, B. A. and R. A. Spicer. 1987. The Evolution and Paleobiology of Land Plants. Croom Helm, London.

Thomas, R. H., W. Schaffner, A. C. Wilson, and S. Pääbo. 1989. DNA phylogeny of the extinct marsupial wolf. Nature 340: 465–467.

Wilson, M. A., B. Gaut, and M. T. Clegg. 1990. Chloroplast DNA evolves slowly in the palm family (Arecaceae). Molec. Biol. Evol. 7: 303–314.

Wolfe, K. H., M. Golly, Y.-W. Yang, P. M. Sharp, and W.-H. Li. 1989. Date of the monocot-dicot divergence estimated from chloroplast DNA sequence data. Proc. Natl. Acad. Sci. U.S.A. 84: 9054–9058.

_____, W.-H. Li, and P. M. Sharp. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc. Natl. Acad. Sci. U.S.A. 84: 9054–9058.

Wu, C. and W.-H. Li. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. Proc. Natl. Acad. Sci. U.S.A. 82: 1741–1745.

Zuckerkandl, E. and L. Pauling. 1965. Evolutionary divergence and convergence in proteins. Pp. 97–166 *in* V. Bryson and H. J. Vogel (eds.), Evolving Genes and Proteins. Academic Press, New York.

NUCLEAR DNA MARKERS FOR THE EVOLUTION OF *MICROSERIS* (ASTERACEAE)[1]

Konrad Bachmann[2]

ABSTRACT. The impact of DNA markers in biosystematic research is illustrated by a survey of recent advances in the biosystematics of the lactucean genus *Microseris*. The genus contains about 13 species that exemplify a wide variety of speciation mechanisms. All but two of these occur only in western North America. The other two are the results of chance long-distance dispersal, each probably involving a single-plant founder event. *Microseris pygmaea* of Chile is a diploid, autogamous annual closely related to the diploid Californian species. Nuclear DNA markers (RFLPs, RAPDs) are being used to reconstruct the stepwise differentiation of the species during its range extension and to analyze the genetics of the phenotypic evolution associated with this dispersal. Comparisons of *M. pygmaea* with diverse populations of its two close Californian relatives, *M. bigelovii* and *M. elegans*, have shown that each of the three species is monophyletic, but the cladistic relationship of the three species cannot be resolved. DNA fingerprint patterns obtained with the oligonucleotide $(GATA)_4$ as probe are hypervariable in *M. pygmaea* but informative about intraspecific evolution and dispersal in *M. elegans*. The widespread and variable perennial *M. scapigera* of Australia and New Zealand is the allotetraploid product of an ancient hybridization between a perennial and an annual North American species, in which the annual ancestor has supplied the chloroplasts. Nuclear RFLPs show that the "annual" genome in *M. scapigera* is not derived from one of the existing species but forms a sister group to the recent annuals. The remarkable adaptive radiation of *M. scapigera* after its arrival in Australia can be reconstructed with nuclear RFLPs.

Molecular methods for the detection of DNA polymorphisms play an increasing role in plant taxonomy (Crawford, 1990; Soltis et al., 1992). For practical reasons, chloroplast DNA (cpDNA) has become the preferred material. Two of the characteristics that recommend cpDNA for taxonomic studies limit its use in biosystematic studies at the population and species level. One of these is the relatively slow rate of sequence evolution. Intraspecific variation in cpDNA can usually be detected in sufficiently thorough surveys (Soltis et al., 1992), but it is not as abundant as are DNA polymorphisms in certain fractions of nuclear DNA. Moreover, the uniparental inheritance of cpDNA without recombination can be very useful for tracing parental lines in reticulate evolution, but it provides no markers for the analysis of the allelic variation and Mendelian inheritance that is central to many biosystematic studies. For this, nuclear DNA polymorphisms are needed. Inevitably, it is technically more difficult to find clearly defined nuclear DNA polymorphisms, and the interpretation of nuclear polymorphisms is not as straightforward as that of polymorphisms in the cpDNA (Bachmann, 1992b). Still, the availability of a virtually unlimited amount of nuclear DNA markers, and the introduction of simpler and less expensive methods to find them, begins to have an impact on biosystematic research. In the following I shall illustrate this with a survey

[1]Support for this research has come from BION, a subsidiary of the Netherlands Organization for Research, NWO, via a postdoc stipend to A. W. van Heusden. This research would have been impossible without the continued advice and practical help of Kenton L. Chambers. We are grateful to Neville Scarlett of LaTrobe University and Barry Sneddon of Victoria University, Wellington, New Zealand, for the Australian material referred to in this paper.

[2]Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands.

of recent advances in our analysis of the evolution within the lactucean genus *Microseris*. Most of the new results concern the genetic relationships among biotypes, populations, and species within the genus, i.e., necessary background information for the analysis of the processes involved in generating this diversity. The importance of this information is underlined by the fact that it has revealed some wrong interpretations of morphological and hybridization data and provided answers where previous data were only suggestive. The new molecular data also allow some conclusions about long-distance gene flow, achene dispersal, and the evolution of morphological and physiological characters of the plants. Eventually, nuclear DNA markers can be used to find, mark, isolate, and analyze the genes involved in speciation and adaptation.

THE GENUS *MICROSERIS*

The small genus *Microseris* is a model system for comparative research on speciation mechanisms including isolation by long-distance dispersal, polyploidy, chromosome restructuring, and genome size changes, and changes in breeding system and ecological adaptation. With two exceptions, all species of *Microseris* occur in western North America and all have a basic chromosome number of $x = 9$. Up to very recently, there were 16 species recognized in the genus (Chambers, 1955), eleven diploid ($2n = 18$) and five tetraploid ($2n = 36$; Stebbins et al., 1953; Chambers, 1955; Oud et al., 1983). Nine of the species are autogamous annuals; seven are obligate outcrossing perennials. *Microseris scapigera* of Australia and New Zealand is essentially an allotetraploid obligate outcrossing perennial, but it contains some octoploid populations and some populations that reproduce by selfing (Sneddon, 1978) or even possibly by agamospermy. In spite of the common chromosome number, the perennials have more DNA per haploid genome than the annuals (Price and Bachmann, 1975), mainly repetitive DNA (Bachmann and Price, 1977; Hemleben et al., 1978), and they are adapted to more mesic conditions (Bachmann et al., 1979). Intraspecific variation in 2C genome size has been documented in *M. douglasii* (Price et al., 1980) and *M. bigelovii* (Price et al., 1981a), and has been correlated with local conditions of soil and climate (Price et al., 1981b, 1986). *Microseris scapigera* contains two small and two large genomes (Chambers, 1955) and therefore may be the allotetraploid hybrid of an annual and a perennial ancestor. Morphology suggests that the perennial ancestor was the arctic-alpine *M. borealis* or a taxon similar to it. Increases in the nuclear DNA amount correlated with invasion of a montane habitat (*M. borealis*) and successive decreases in genome size correlated with adaptation to dry grassland (via *M. lindleyi* to the small annuals) could easily be fitted into the phylogenetic scheme underlying the taxonomic treatment by Chambers (Price and Bachmann, 1975; Bachmann, 1987).

All species of *Microseris* have patchy distributions in more or less isolated local populations with much variation among and little variation within populations. *Microseris scapigera* seems to have been abundant over wide areas in Australia before the advent of cattle and sheep (Gott, 1983). Its present rarity is of recent origin, but the population structure of the Californian species seems to be the result of the patchy distribution of sites with the proper conditions of soil type and moisture in California (Chambers, 1955). This is extreme in some of the autogamous annuals in which a local population often consists of one genetically rather uniform "biotype." These biotypes may vary from one population to the next so that it is difficult to find morphological character states common to all plants of a species and absent from the other species. We have raised a great many F1 hybrids between plants from different populations of one species and between plants from different species. In each case, intraspecific crosses were highly fertile. Interspecific hybrids are rarely completely sterile, but always show measurably reduced fertility (Chambers, 1955; Bachmann and

Chambers, unpublished). We can therefore assume that the species as recognized by Chambers (1955) are natural biological entities, even if they are difficult to define by simple morphological characters. The identity of these species is threatened on one hand by the geographic isolation of populations and on the other hand by the frequent coexistence of several species at a suitable site and the partial fertility of interspecific hybrids.

CHLOROPLAST DNA

The impact of molecular data became evident when Wallace and Jansen (1990) examined restriction site variation for 22 restriction endonucleases in the chloroplast DNA of all 16 species. Their results show that chloroplast DNA of the annual *M. lindleyi* is clearly differentiated by 57 mutations from that of all other species of the genus, which form two distinctive clusters, one supported by 19 synapomorphic mutations containing all diploid perennials, the other supported by 24 mutations containing all diploid annuals together with the chloroplasts of all tetraploids. Little variation was found within either of these two groups. It may be significant that the chloroplast DNA of the Australian *M. scapigera* seems to be the least derived among the annuals. The data not only place this disjunct species clearly into *Microseris*, they also support the suggestion by Chambers (1955) that an annual has contributed a genome to the original hybrid from which the perennial *M. scapigera* is derived.

The distinct position of *M. lindleyi* was unexpected but plausible. *Microseris lindleyi* differs morphologically and in its intermediate genome size from the other annuals (Bachmann et al., 1979). However, the existence of two allotetraploid species combining genomes of *M. lindleyi* with those of other annuals had suggested a close genetic relationship (Irmler et al., 1983). In both hybrid combinations (*M. heterocarpa* with *M. douglasii* and the rare *M. decipiens* with *M. bigelovii*), *M. lindleyi* is the pollen donor. *Microseris heterocarpa* shares chloroplast DNA variation with *M. douglasii* and seems to have originated from several independent hybridizations (Wallace and Jansen, 1989).

A more inclusive study of chloroplast DNA variation has shown that *M. lindleyi* must be excluded from *Microseris* altogether (Jansen et al., 1991). This seems to be the first instance of a formal taxonomic revision based on molecular data. *Microseris lindleyi* now forms the monotypic genus, *Uropappus*, and a new genus, *Stebbinsoseris*, has been erected by Chambers for its allotetraploid hybrids with annual species of *Microseris* (Jansen et al., 1991; Figure 1).

As in several other cases where molecular data disagree with current taxonomic treatment, there are two conclusions from this result: (1) it is virtually impossible to resolve conflicting phenotypic evidence by estimating the relative weight of the characters, and (2) arranging phenotypic character states in trends of increasing specialization, as I did with the genome size values (Bachmann, 1987), can lump several independent evolutionary lines into a plausible single sequence. Since both effects can occur even in a strictly cladistic treatment of phenotypic data, a cladistic approach in itself is no safeguard against this source of error.

MICROSERIS PYGMAEA: LONG-DISTANCE DISPERSAL AND FOUNDER EVENTS

Besides *M. scapigera*, only the small diploid annual *M. pygmaea* of central Chile occurs outside western North America. Both species have probably arisen after long-distance transport of a single achene from western North America, in one case to Australia, in the other to Chile. *Microseris pygmaea* is one of more than a hundred cases of "amphitropical" disjunct distributions between regions with mediterranean climate in North and South America (Raven, 1963; Chambers, 1963; Carlquist, 1981, 1983). Since all *M. pygmaea* differ

from all other annuals of the genus by having a pappus of 10 rather than 5 or fewer paleaceous parts, and since this character has a complex genetic basis (Chambers, 1963; Bachmann et al., 1982, 1985a), an origin of *M. pygmaea* in Chile from a single achene is likely. The species has progressively changed during its spread into inland Chile, and there are indications that this evolution has involved subsequent founder events and the homozygous fixation of new mutations (Bachmann et al., 1985a). If this hypothesis is correct, we should be able to construct a cladogram of the populations within the species. The enzyme locus GOT-2 (glutamate-oxalacetate transaminase = aspartate aminotransferase) illustrates this clearly. All North American plants of the genus are homozygous for the same allele at this locus. This allele is also found in three coastal populations of *M. pygmaea*, while all inland populations are homozygous for another allele of GOT-2 (Bachmann, 1983). Some other isoenzyme loci are also fixed in alternate homozygous genotypes in various populations (Bachmann et al., 1985b).

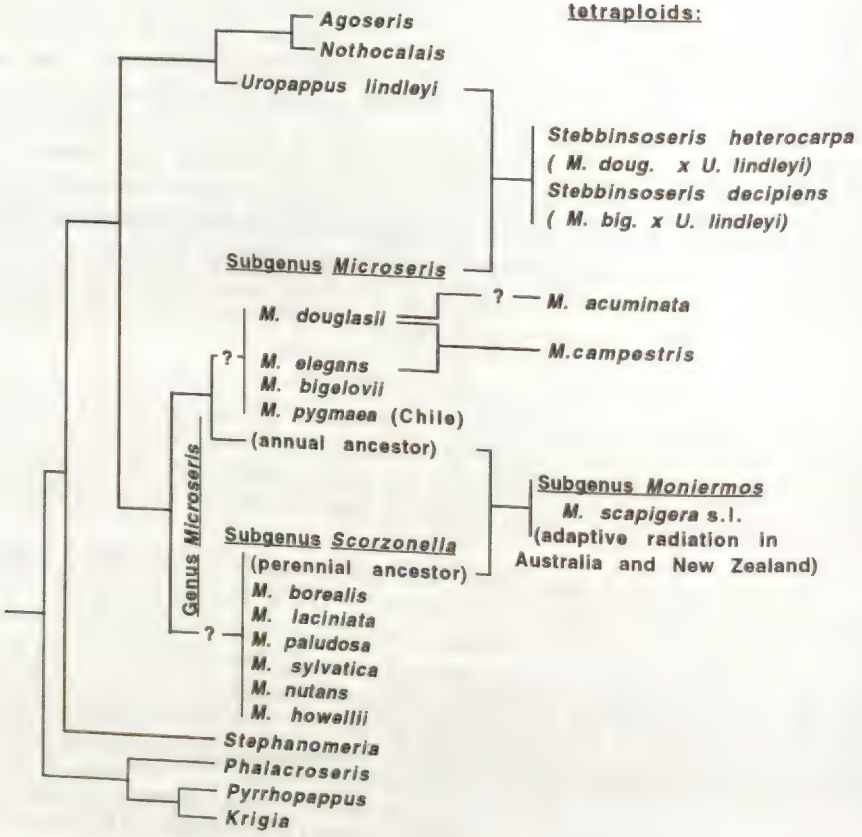


Figure 1. Phylogenetic position of the genus *Microseris* and its constituent species after the most recent revision incorporating molecular data (Jansen et al., 1991). Unresolved phylogenetic questions (question marks) concern the relationships among the diploid annuals, the diploid perennials, and the ancestry of the tetraploid *M. acuminata*. *Microseris scapigera* of Australia and New Zealand consists of several taxa which may deserve specific status (Sneddon, 1978).

We have tried to reconstruct the genetic events underlying the phenotypical evolution of *M. pygmaea* by analyzing crosses among plants from various populations with

a common tester strain of the North American *M. bigelovii* (Bachmann et al., 1985a; van Heusden, 1990) and the segregation of an intraspecific hybrid involving the morphologically most distinct biotypes of *M. pygmaea* (van Houten and Bachmann, in preparation). This has shown that the phenotypic changes cannot be assembled into a population cladogram without permitting parallel mutations with identical phenotypic effects. When methods for the analysis of nuclear DNA variation became available we employed these for an independent test of the intraspecific evolution in *M. pygmaea*.

RFLPs, DNA FINGERPRINTS, RAPDs

We have used three different approaches to find sequence variation in the nuclear DNA:

(1) Cloned pieces of single-copy and repetitive nuclear DNA of *M. pygmaea* were used as probes to detect restriction fragment length polymorphisms, RFLPs (van Houten et al., 1993). The considerable amount of screening necessary to assemble a set of informative probes and the relatively low intraspecific variability for restriction fragment lengths in *M. pygmaea* make this a very expensive and time-consuming approach.

(2) Multiple-band DNA fingerprints were obtained when restricted DNA was probed with synthetic simple sequence oligonucleotides, e.g., $(GATA)_4$ (Weising et al., 1989). Within one population of *M. pygmaea*, few individuals had identical fingerprint patterns, the patterns changed in an unpredictable way from parent plants to their offspring by selfing, and the inheritance of fingerprint bands in an interpopulation F2 did not always follow Mendelian expectations (van Houten et al., 1991). While hypervariability is a well-known feature of DNA fingerprint alleles (Jeffreys et al., 1985), few plants have shown the amount of variability seen in *M. pygmaea* (Schaal et al., 1991). In the related species, *M. elegans*, fingerprint patterns obtained with the same method were characteristic for biotypes and informative about the relationship among populations (van Heusden and Bachmann, 1992a). The hypervariability of fingerprint patterns in *M. pygmaea* also contrasts with the complete uniformity of a complex banding pattern throughout the species obtained when restricted nuclear DNA was probed with a cloned piece of repetitive DNA (van Houten, unpublished).

(3) Nuclear DNA was amplified in vitro with the polymerase chain reaction from arbitrary short primers (Welsh and McClelland, 1990; Williams et al., 1990). The method depends on the accidental presence of sequences complementary to the primers in both strands of the double-stranded DNA at a distance of a few hundred base-pairs, which permits the amplification of the DNA between the two primer binding sites. Sequence variation, such as a mutation in one of the primer sites, prevents DNA amplification and is recognized by the absence of a specific amplification product (random amplified polymorphic DNA: RAPD, Williams et al., 1990). As in multi-locus DNA fingerprinting, bands at one position in a gel after electrophoresis are assumed to be identical. Misinterpretations are possible. Since the amplification reaction accepts DNA from any source as template, the plant material and the DNA extracted from it must be reliably clean. The method is sensitive to various kinds of error, but once it has been standardized it can provide a very large number of markers, and the reliability of the results can be monitored by appropriate controls (Hadrys et al., 1992).

RAPD VARIATION IN *MICROSERIS PYGMAEA*

We have amplified DNA from 9 inbred strains derived from 8 populations representing the range of variation found in *M. pygmaea* (van Heusden and Bachmann, 1992c). Some 208 amplification products were obtained with 24 primers. Ninety-one of these were shared by all strains; 93 were informative. Parsimony analysis was carried out using

PAUP version 3.0 developed by D. L. Swofford (Swofford and Olsen, 1990). The shortest cladistic tree had 140 steps and a consistency index of 0.686. It confirms the dichotomy between the coastal and the inland strains, which previously had been distinguished by their alleles for GOT-2, by morphology and by differences in flowering time. The branching topology for the inland strains shows the predicted pattern of successive stepwise colonizations. However, when the bootstrap option was used to run 100 replicates to obtain confidence intervals (Felsenstein, 1985), little besides the clear distinction between coast and inland populations proved reliable. Apparently the populations are not as isolated from each other as the homozygous isoenzyme loci had suggested, and there seems to be some gene flow and recombination among neighboring populations. We have estimated homoplasy in the RAPD data due to all possible sources, including faults in band interpretation, at about 10% (van Heusden and Bachmann, 1992c), and many of the polymorphisms between the coastal and inland strains of *M. pygmaea* have meanwhile been mapped by segregation analysis in an interpopulation hybrid (van Houten, unpublished).

INCONCLUSIVE SEARCH FOR THE CALIFORNIAN ANCESTOR OF *M. PYGMAEA*

If the ancestor of *M. pygmaea* has come from California, it is more likely to be related to either *M. elegans* or *M. bigelovii* than to *M. douglasii* among the diploid annuals. Morphological characters are ambiguous about the relationship among the three species. Chambers (1955) suggests that *M. elegans* and *M. bigelovii* are more closely related to each other than either is to *M. pygmaea*. A considerable increase in our knowledge of genetic variation in the three species has not brought us much further. I have mentioned above that the genetic differentiation among populations in any of the species leaves few species-specific characters for such an analysis.

We have selected the two genetically most different strains available from each of the three species and have investigated their relationship on the basis of RAPDs obtained with 22 primers (van Heusden and Bachmann, 1992c). Of 143 amplification products, 49 were shared by all 6 strains. Since RAPDs are mainly known for their variability, I want to emphasize that the method also yields amplification products that are uniform across larger taxonomic units and from which probes for taxonomic analysis at higher levels may be developed. Of the products, 32 were species-specific, 6 for *M. elegans*, 12 for *M. bigelovii* and 14 for *M. pygmaea*; 12 of the products were present in all but one strain, and 50 amplification products were informative. The distribution of these was essentially random, and there was no indication of a closer association between two of the three species. Of the three possible pairwise combinations of species, each was supported by 5 synapomorphic RAPDs. The cladistic analysis therefore supports the monophyly of each of the three species but leaves a completely unresolved trichotomy for their relationship. The dozens of characters produced by the RAPD method have not helped at all in our search for the Californian ancestor of *M. pygmaea*. The method has, however, produced a whole series of species-specific amplification products. Simple species-specific characters are at a premium in the annual species of *Microseris*. The ease with which the distribution of a great number of character states can be screened makes the RAPD method extremely valuable. Since the amplification products can be cloned, the method can be used to identify and characterize selected polymorphisms.

MICROSERIS ELEGANS AND *M. BIGELOVII*

Microseris bigelovii has a very striking geographical distribution along the Pacific coast from Pt. Sal in southern California to mid-Oregon, with a disjunct occurrence on

Vancouver Island, B.C. (Chambers, 1955). *Microseris elegans* occurs in the interior valleys and foothills throughout central California, and replaces *M. bigelovii* at the coast from Los Angeles south to Baja California and on the Channel Islands. Both species show the typical distribution in isolated populations depending on the availability of sites with the proper conditions of soil and climate (Chambers, 1955). We have analyzed the genetic relationships among a representative collection of biotypes from either species with RAPDs and found subtle but interesting differences.

Of the three species which we have compared up to now, *M. elegans* comes closest to the model of colonization of new sites by founder events (van Heusden and Bachmann, 1992a). The distribution of RAPDs among populations of this species could be compared with that of DNA fingerprint bands and that of a few isoenzyme alleles. The results of all three methods were congruent. The biotypes of this species belong to three monophyletic groups with apparently little gene exchange. One of these groups combines the three northernmost populations in our sample, while the biotypes forming the other two groups come from populations that are not nearest neighbors. The pattern suggests that very rarely an achene from one population gets transported, possibly over a distance of a few hundred kilometers, to an unoccupied suitable site where it starts a population that is closely related to its distant population of origin but genetically isolated from a much nearer population of the same species.

A cladistic treatment of the RAPD data for biotypes of *M. bigelovii* (van Heusden and Bachmann, 1992b) recognizes only geographically nearest neighbors as monophyletic groups. One such group comprises plants from three populations on southern Vancouver Island and supports our suggestion that this disjunct occurrence is the result of one chance introduction. Beyond these near neighbor groups, character distributions among populations are nearly random. We interpret this as an indication of a low level of gene flow by achene transport among these populations. Occasional long-distance achene transport along the coastal distribution area of *M. bigelovii* may be more frequent than in the inland area of *M. elegans* and more likely to deposit an achene in an already occupied site, thereby initiating a burst of recombination followed by selection and/or drift.

The difference between a correlated distribution of characters in *M. elegans* and a randomization of character associations in *M. bigelovii* can also be recognized in the distribution of morphological characters (Bachmann et al., 1984; Bachmann and van Heusden, 1992; Bachmann, 1992a).

CHANCE EVENTS: *MICROSERIS SCAPIGERA*

The dispersal events that we postulate in order to explain the character distributions within and among species of *Microseris* must all be rare occurrences. There has been only one single successful introduction into Chile, and the North American populations either are completely isolated or receive immigrants so rarely that most of them are genetically uniform and distinct. A very low rate of gene exchange among populations, far too rare to be directly observed, may be sufficient to randomize the character associations in *M. bigelovii*. It is difficult to decide between rare but regular occurrences and true chance events with a probability so low that their occurring even once cannot be expected. The colonization of southern Vancouver Island by *M. bigelovii*, the colonization of Chile by *M. pygmaea*, and the colonization of Australia and New Zealand by *M. scapigera* are certainly chance dispersal events. In the case of *M. scapigera*, a concurrence of several very unlikely events must have taken place (Bachmann, 1987). This includes the initial hybridization between an annual and a perennial species. When we repeated this, the sterile diploid hybrids were annuals

(Chambers and Bachmann, unpublished) and died before we could induce polyploidy. Polyploidization, the transport of an achene to Australia and the establishment of a population are all unlikely events. Still, RFLP data both on chloroplast DNA (Wallace and Jansen, 1990) and on nuclear DNA (van Houten et al., 1993) confirm the hypothesis of Chambers (1955) about the allopolyploid origin of *M. scapigera*. The nuclear DNA data agree with the chloroplast DNA data in suggesting that the annual ancestor of *M. scapigera* originated before the formation of the modern annuals.

These data are still incomplete, but they suggest an answer to one of the improbable aspects of *M. scapigera*. With few exceptions, plants of *M. scapigera* are obligate outcrossers. A single isolated achene of a self-incompatible species would not be able to start a population. Conversely, it is very unlikely that the ancestor of *M. scapigera* has reached Australia other than as a single propagule. We have recently obtained material from two Australian populations in which isolated plants set abundant seed either by selfing or possibly by agamospermy (N. Scarlett, La Trobe University, personal communication). The morphology of the plants had suggested to us that they represent a derived type that has secondarily abandoned self-incompatibility. Interestingly, these plants appear to be primitive on the basis of nuclear RFLPs (van Houten et al., 1993), and may have preserved the original reproductive mode. We are now investigating this possibility and the corollary that the impressive adaptive radiation of this species in Australia, Tasmania, and New Zealand may have followed a switch to obligate outcrossing.

CONCLUSION AND OUTLOOK

The last example underlines the value of independent molecular data on the phylogeny for the analysis of evolutionary mechanisms. The hypothesis that obligate outcrossing in *M. scapigera* is a derived condition is based entirely on DNA data. The consequence of this for the interpretation of the evolution of this species is enormous. Fortunately, the material for a detailed experimental investigation is available.

DNA data have greatly advanced our understanding of the evolution of *Microseris* in a relatively short time. This, however, is only a beginning, and the questions that now need to be answered follow logically from the results reported here. The phylogenetic relationships of the annual and the perennial species can be analyzed with nuclear markers. The problems we have encountered with the relationship among *M. elegans*, *M. bigelovii*, and *M. pygmaea* may find their solution when the fourth diploid annual, *M. douglasii*, is included in the analysis. This is a very variable species, and we are only now developing the representative collection of strains needed for the comparison. The few RFLPs found by Wallace and Jansen (1989, 1990) among the annuals suggest the possibility of a more complex relation among the four diploid annuals than we assume now. The possibility of introgression between *M. douglasii* and other species, which has been mentioned by Chambers (1955), can now be investigated. Phylogenetic analysis with DNA markers can contribute much essential information.

Up to now, molecular and phenotypic data have been obtained independently in parallel investigations. Our investigation of the phenotypic evolution in *Microseris* has involved a considerable amount of quantitative genetic analysis of segregating hybrid progeny. We have lately tested the segregation of nuclear DNA polymorphisms, both RFLPs and RAPDs, and regularly find cosegregation between a monogenic molecular marker and one of the many simultaneous quantitative character segregations in an interpopulation or interspecific hybrid. The detection by Vlot et al. (1992) of a major gene permitting deviations of pappus part numbers from the basic number of 5 in *M. douglasii* was greatly helped by cosegregation with an RFLP. With this approach, molecular and phenotypic analyses converge, and we may eventually succeed in tracing the molecular changes responsible for natural phenotypic variation (Doebley, 1994).

LITERATURE CITED

Bachmann, K. 1983. Genetic analysis of evolution associated with dispersal in plants. Sonderb. Naturwiss. Vereins Hamburg 7: 65–86.

_____. 1987. Speciation in *Microseris*. Pp 67–80 *in* P. Hovekamp (ed.), Systematics and Evolution: A Matter of Diversity. Utrecht University.

_____. 1992a. Phenotypic similarity and genetic relationship among populations of *Microseris bigelovii* (Asteraceae: Lactuceae). Bot. Acta 105: 337–342.

_____. 1992b. Nuclear DNA markers in angiosperm taxonomy. Acta Bot. Neerl. 41: 369–384.

_____, K. L. Chambers, J. Grau, and H. J. Price. 1985b. Genetic variation in *Microseris pygmaea* (Asteraceae-Lactuceae). Beitr. Biol. Pflanzen 60: 51–88.

_____, _____, and H. J. Price. 1979. Genome size and phenotypic evolution in *Microseris* (Compositae, Cichoriaceae). Pl. Syst. Evol. Suppl. 2: 41–66.

_____, _____, and _____ . 1984. Differential geographic distribution of spatulate and pointed leaf shapes in *Microseris bigelovii* (Asteraceae–Lactuceae). Beitr. Biol. Pflanzen 59: 5–14.

_____, _____, _____, and A. König. 1982. Four additive genes determining pappus part numbers in *Microseris* annual hybrid C34 (Asteraceae, Lactuceae). Pl. Syst. Evol. 141: 123–141.

_____ and H. J. Price. 1977. Repetitive DNA in Cichorieae (Compositae). Chromosoma 61: 267–275.

_____ and A. W. van Heusden. 1992. Phenotypic similarity and genetic relationship in *Microseris elegans* (Asteraceae: Lactuceae). Biol. Zentralbl. 111: 38–53.

_____, _____, K. L. Chambers, and H. J. Price. 1985a. Duplication of additively acting genes in the evolution of a plant (*Microseris pygmaea*). Experientia (Basel) 41: 1348–1350.

Carlquist, S. 1981. Chance dispersal. Amer. Sci. 69: 509–516.

_____. 1983. Intercontinental dispersal. Sonderb. Naturwiss. Vereins Hamburg 7: 37–47.

Chambers, K. L. 1955. A biosystematic study of the annual species of *Microseris*. Contrib. Dudley Herb. 4: 207–312.

_____. 1963. Amphitropical species pairs in *Microseris* and *Agoseris* (Compositae: Cichorieae). Quart. Rev. Biol. 38: 124–140.

Crawford, D. J. 1990. Plant Molecular Systematics: Macromolecular Approaches. Wiley Interscience, New York.

Doebley, J. 1994. Genetics, development, and the morphological evolution of maize. (This volume.)

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Gott, B. 1983. Murnong—*Microseris scapigera*: a study of a staple food of Victorian aborigines. Austral. Aboriginal Stud. 1983: 2–18.

Hadrys, H., M. Balick, and B. Schierwater. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molec. Ecol. 1: 55–63.

Hemleben, V., K. Bachmann, and H. J. Price. 1978. Ribosomal gene numbers in Microseridinae (Compositae: Cichorieae). Experientia (Basel) 34: 1452–1453.

Irmler, C., K. Bachmann, K. L. Chambers, H. J. Price, and A. König. 1983. Enzymes and quantitative morphological characters compared between the allotetraploid *Microseris decipiens* and its diploid parental species. Beitr. Biol. Pflanzen 57: 269–289.

Jansen, R. K., R. S. Wallace, K.-J. Kim, and K. L. Chambers. 1991. Systematic implications of chloroplast DNA variation in the subtribe Microseridinae (Asteraceae: Lactuceae). Amer. J. Bot. 78: 1015–1027.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985. Hypervariable 'minisatellite' regions in human DNA. Nature 314: 67–73.

Oud, J. L., F. Schuring, and C. Berg. 1988. The karyotypes of *Microseris bigelovii*, *M. douglasii*, and *M. pygmaea* (Asteraceae). Pl. Syst. Evol. 159: 249–256.

Price, H. J. and K. Bachmann. 1975. DNA content and evolution in the Microseridinae. Amer. J. Bot. 62: 262–267.

_____, _____, K. L. Chambers, and J. Riggs. 1980. Detection of intraspecific variation in nuclear DNA content in *Microseris douglasii*. Bot. Gaz. 141: 195–198.

_____, K. L. Chambers, and K. Bachmann. 1981a. Genome size variation in diploid *Microseris bigelovii* (Asteraceae). Bot. Gaz. 142: 156–159.

_____, _____, and _____. 1981b. Geographic and ecological distribution of genomic DNA content variation in *Microseris douglasii* (Asteraceae). Bot. Gaz. 142: 415–426.

_____, _____, _____, and J. Riggs. 1986. Patterns of mean nuclear DNA content in *Microseris douglasii* (Asteraceae) populations. Bot. Gaz. 147: 496–507.

Raven, P. H. 1963. Amphitropical relationships in the floras of North and South America. Quart. Rev. Biol. 38: 151–177.

Schaal, B. A., S. L. O'Kane, Jr., and S. H. Rogstad. 1991. DNA variation in plant populations. Trends Ecol. Evol. 6: 329–333.

Sneddon, B. V. 1978. A biosystematic study of *Microseris* subgenus *Monermos* (Compositae, Cichorieae). Dissertation, Victoria University, Wellington, New Zealand.

Soltis, P. S., D. E. Soltis, and J. J. Doyle. 1992. Molecular Systematics of Plants. Chapman and Hall, New York.

Stebbins, G. L., J. A. Jenkins, and M. S. Walters. 1953. Chromosomes and phylogeny in the Compositae, tribe Cichorieae. Univ. Calif. Publ. Bot. 26: 401–430.

Swofford, D. L. and G. J. Olsen. 1990. Phylogeny reconstruction. Pp. 411–501 *in* D. M. Hillis and C. Moritz (eds.), Molecular Systematics. Sinauer, Sunderland, MA.

Van Heusden. A. W. 1990. Genetic analysis of natural variation in *Microseris pygmaea* (Asteraceae) in crosses with *M. bigelovii* C93b. Dissertation, University of Amsterdam, The Netherlands.

_____ and K. Bachmann. 1992a. Genotype relationships in *Microseris elegans* (Asteraceae, Lactuceae) revealed by DNA amplification from arbitrary primers (RAPDs). Pl. Syst. Evol. 179: 221–233.

_____ and_____. 1992b. Nuclear DNA polymorphisms among strains of *Microseris bigelovii* (Asteraceae, Lactuceae) amplified from arbitrary primers. Bot. Acta 105: 331–336.

_____ and_____. 1992c. Genetic differentiation in *Microseris pygmaea* (Asteraceae, Lactuceae) studied with DNA amplification from arbitrary primers (RAPDs). Acta Bot. Neerl. 41: 385–395.

Van Houten, W. H. J., A. W. van Heusden, J. Rouppe van der Voort, L. Raijmann, and K. Bachmann. 1991. Hypervariable DNA fingerprint loci in *Microseris pygmaea* (Asteraceae, Lactuceae). Bot. Acta 104: 169–256.

_____, N. Scarlett, and K. Bachmann. 1993. Nuclear DNA markers of the Australian tetraploid *Microseris scapigera* and its North American diploid relatives. Theor. Appl. Genet. 87: 498–505.

Vlot, E. C., W. H. J. van Houten, S. Mauthe, and K. Bachmann. 1992. Genetic and non-genetic factors influencing deviations from five pappus parts in a hybrid between *Microseris douglasii* and *M. bigelovii* (Asteraceae, Lactuceae). Int. J. Pl. Sci. 153: 89–97.

Wallace, R. S. and R. K. Jansen. 1989. DNA evidence for the origin of allopolyploid annual species of *Microseris* (Asteraceae: Lactuceae). Amer. J. Bot. 76 Suppl.: 280 (abstract).

_____ and _____ . 1990. Systematic implications of chloroplast DNA variation in the genus *Microseris* (Asteraceae: Lactuceae). Syst. Bot. 15: 606–616.

Weising, K., F. Weigand, A. J. Driesel, G. Kahl, H. Zischler, and J. Epplen. 1989. Polymorphic simple GATA/GACA repeats in plant genomes. Nucl. Acids Res. 17: 10128.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18: 7213–7218.

Williams, J. G. K., A. R. Kubelik, K. J. Litvak, J. A. Rafalsky, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531–6535.

A PHYLOGENY OF SEED PLANTS RESULTING FROM ANALYSIS OF DNA SEQUENCE VARIATION AMONG THE *rbc*L LOCI OF 499 SPECIES, WITH PARTICULAR EMPHASIS ON ALLIANCES AMONG MONOCOTYLEDONS[1]

Melvin R. Duvall[2,3], Mark W. Chase[4],
Douglas E. Soltis[5], and Michael T. Clegg[2]

ABSTRACT. DNA sequences of the plastid locus that encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) have been determined for over 500 species of seed plants, including over 100 monocotyledons, with representative species from all major taxa. These data were analyzed by the maximum likelihood method and a parsimony method. The resulting phylogenetic trees identify major lineages of seed plants that are largely in agreement with traditional taxonomic schemes. Within those lineages, however, certain taxa appear to be paraphyletic. Two problematic genera, *Ceratophyllum* and *Acorus*, were found to occupy unique, basal positions within the angiosperms and the monocotyledons, respectively. This broad analysis of seed plants suggests that more intensive infraordinal or infrafamilial sampling of *rbc*L sequence data may clarify relationships within selected taxa.

Martin and Dowd (1989) recently summarized the state of angiosperm systematics with the following: "... four important systems of classification have been published [Takhtajan (1980), Cronquist (1981), Thorne (1981), and Dahlgren (1983)] ... and the disagreements between them are such that at least three of them, probably all four, must be wrong in major ways." Resolution of higher-order relationships among flowering plants requires the recognition of phylogenetically meaningful homologies between long-diverged species. The advent of molecular systematics offers a new approach to the determination of relationships, and analysis of molecular characters has the potential to identify ancient homologies.

The most abundant protein on earth is ribulose-1,5,-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco is a key enzyme in photosynthetic carbon metabolism and is thus ubiquitous among green plants. The large subunit of Rubisco is encoded by the plastid locus, *rbc*L. This locus, like the entire plastid genome, is highly conserved in an evolutionary sense so that the alignment of sequences of *rbc*L from long-diverged species is a straightforward matter. The phylogenetic utility of *rbc*L data among restricted groups of seed plants has been demonstrated for: (1) Poaceae (Doebley et al., 1990); (2) fossil *Magnolia* and *Taxodium* (Golenberg et al., 1990; Soltis et al., 1992, respectively); (3) Arecaceae (Wilson et al., 1990); (4) Dipsacales (Donoghue et al., 1992); (5) the polyphyletic carnivorous plant groups (Albert et

[1]We thank R. Olmstead, D. Jarrell, and others for unpublished *rbc*L sequences; J. F. Smith and N. Stone, who each provided DNA from one species; J. C. French who provided DNA extracts for Araceae; and W. Armstrong for identification of one species. For computer analyses we thank G. Learn, G. J. Olson, R. Overbeek, and the Concurrent Supercomputing Consortium. This work was supported by grants from National Science Foundation (BSR-9002321) to M. Duvall and National Institutes of Health (GM45144) to M. Clegg.

[2]Department of Botany and Plant Sciences, University of California, Riverside, California 92521, U.S.A.
[3]Present Address: Department of Biology and Microbiology, South Dakota State University, Brookings, South Dakota 57007, U.S.A.
[4]Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599, U.S.A. Present address: Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, U. K.
[5]Department of Botany, Washington State University, Pullman, Washington 99164, U.S.A.

al., 1992); (6) Caryophyllanae (Giannasi et al., 1992); (7) Asteraceae (Kim et al., 1992); and (8) Asteridae (Olmstead et al., 1992), among other taxa (see papers in *Annals of the Missouri Botanical Garden* 80 (3)). Our goal here was to reconstruct higher-order phylogenetic relationships among the angiosperms with special focus on the monocotyledons using DNA sequence data from the *rbc*L loci of many species that represent much of the breadth of diversity in the group.

MATERIALS AND METHODS

DNA sequences for *rbc*L were determined by different, laboratory-specific procedures, one of which is given here. Total DNA was extracted from fresh leaf tissue by one of several methods (Palmer, 1986; Doyle and Doyle, 1987; Jarrell et al., 1992). Approximately 1 mg of each DNA preparation was used to provide template for *Taq*-mediated amplification of *rbc*L using the protocol provided with *Taq* DNA polymerase by the supplier (Promega Corp.). Two highly conserved sequences from the *rbc*L coding region of *Spinacia oleracea* were used as primers for the reaction. The 5′ primer consisted of the first 30 base-pairs of that sequence and the 3′ (reverse) primer corresponded to positions 1351–1380 on the complementary strand. Amplifications utilizing these primers produced 1320 base pairs of the coding sequence of *rbc*L.

Single-stranded DNA was produced in a second round of amplification using the double-stranded product as template and the two primers individually. These single-stranded products were precipitated (7.5% polyethylene glycol-8000, 0.94 M NaCl), the DNA pellets were washed first with 70% ethanol, then with 95% ethanol, and sequencing was accomplished by the dideoxynucleotide chain-termination method using a set of conserved, synthetic, internal *rbc*L primers (obtained from G. Zurawski, DNAX Corp.). Both the *rbc*L-coding strand and the complementary strand were sequenced for all species. Twenty-nine species of monocotyledons for which *rbc*L sequences were determined by M. Duvall, together with voucher information and GenBank accession numbers, are listed in Table 1.

Nucleotide sequences were encoded as character state data and analyzed by one of two phylogenetic methods. The maximum likelihood method (Felsenstein, 1981) was selected because it is less biased by heterogeneous substitution rates that have been observed for *rbc*L among different lineages of monocotyledons (Gaut et al., 1992) than are other methods. Furthermore, under this method, likelihood scores may be calculated for alternate topologies and subjected to comparative tests of statistical significance. However, computer limitations precluded analysis of the entire *rbc*L data base. A subset of 73 monocotyledonous species was selected for this analysis. *Saururus cernuus* was chosen as an appropriate available dicotyledonous outgroup species based on previous non-molecular phylogenetic studies of the angiosperms (Donoghue and Doyle, 1989). The analysis was executed on the Touchstone Delta Parallel Processing Supercomputer, the fastest existing computer, using the program "fast-DNAml version 1.0.3" (Olsen et al., 1992). This analysis was conducted following the strategy described in Duvall et al. (1993).

Parsimony was selected as a less computer-intensive method for analysis of larger data sets. A subset of 116 monocotyledonous species was analyzed with 16 outgroup species suggested by the work of Donoghue and Doyle (1989), for a total of 132 species. Parsimony was implemented with PAUP Version 3.0s (Swofford, 1990) on a Macintosh IIfx. A heuristic search was performed with ten replications of the input order and global swapping (TBR or "tree-bisection and reconnection"), saving only the first 150 trees (length 4180). Weights were then applied to the 150 initial trees. These weights incorporate *a priori* estimates of transition/transversion ratio and codon position substitution percentages with a correction for multiple substitutions (Albert et al., 1993; Table 2). By this method, one minimal-length tree was selected. Over 15.9 million rearrangements of this tree (TBR swapping) were performed, to arrive at a set of equally parsimonious trees.

Table 1. Twenty-nine species for which *rbc*L was sequenced for this study. Identities for the remaining 104 species that were analyzed may be found in Chase et al. (1993). Classification system used is as described in Results section.

Taxa	Vouchers (Herbaria)	Family	GenBank Accession
ARANAE			
ARALES			
Acorus calamus L.	French 232 (CH)	Araceae	M91625
Gymnostachys anceps R. Br.	Howard 4325 (FTG)	Araceae	M91629
Pistia stratiotes L.	French 233 (CH)	Araceae	M96963
Lemna minuscula Herter	Duvall 19920501 (UCR)	Lemnaceae	M91630
BROMELIANAE			
TYPHALES			
Typha latifolia L.	Bradley 24974 (GMUF)	Typhaceae	M91634
Sparganium americanum Nutt.	Chase 257 (NCU)	Sparganiaceae	M91633
COMMELINANAE			
COMMELINALES			
Commelina benghalensis L.	Duvall 19920603 (UCR)	Commelinaceae	L05033
Tradescantia aff. *pallida*	Bradley 24980 (GMUF)	Commelinaceae	L05041
Tradescantia zebrina hort. ex Bosse.	Bradley 24980 (GMUF)	Commelinaceae	L05042
CYPERALES			
Cyperus alternifolius L.	Duvall 1992002 (UCR)	Cyperaceae	M91627
POALES			
Bambusa multiplex (Lour.) Rausch.	Sanders 62-616 (UCR)	Poaceae	M91626
Zizania texana Hitchc.	Duvall (MIN)	Poaceae	L05043
Joinvillea plicata Hook. f.	L. B. Thren 84 (NO)	Joinvilleaceae	L01471
CYCLANTHANAE			
CYCLANTHALES			
Ludovia integrifolia	Cullina 102679 (CONN)	Cyclanthaceae	--
LILIANAE			
ASPARAGALES			
Aloe vera (L.) Burm.	Bradley 24977 (GMUF)	Asphodelaceae	L05029
Asparagus officinalis L.	Duvall 19920604 (UCR)	Asparagaceae	L05028
Chlorophytum comosum (Thunb.) Jacques	Bradley 7331 (GMUF)	Anthericaceae	L05031
Clivia miniata Reg.	Bradley 24976 (GMUF)	Amaryllidaceae	L05032
Danae racemosa Moench	Sanders 12293 (UCR)	Ruscaceae	L05034
Haworthia subfasciata Baker	Bradley 24978 (GMUF)	Asphodelaceae	L05035
Hemerocallis fulva L.	Duvall 19920601 (UCR)	Hemerocallidaceae	L05036
Ledebouria socialis (Bak.) Jessop	--	Hyacinthaceae	L05038
Lomandra longifolia Labill.	Duvall 19920902 (UCR)	Dasypogonaceae	L05039
Nolina (*Beaucarnea*) *recurvata* (Lem.) Hemsl.	Peterson (US)	Nolinaceae	L05030
LILIALES			
Iris X *germanicum* L.	Bradley 25976 (GMUF)	Iridaceae	L05037
Medeola virginiana (L.) Merrill.	Bradley 24972 (GMUF)	Uvulariaceae	M91613
PANDANAE			
PANDANALES			
Pandanus veitchii Mast. & Moore	Duvall 19920301 (UCR)	Pandanaceae	M91632
ZINGIBERANAE			
ZINGIBERALES			
Maranta leuconeura E. Morr.	Bradley 24979 (GMUF)	Marantaceae	L05040
Hedychium gardnerianum Ker-Gawl	Bradley 24975 (GMUF)	Zingiberaceae	M91628

Table 2. Empirically determined weights applied to nucleotide substitutions used in parsimony analysis. These weights incorporate estimates of transition/transversion ratio and codon position substitution percentages with a correction for multiple substitutions (Albert et al., 1993).

Codon Position	Transition	Transversion
1	5520	6620
2	6368	7470
3	4039	5127

The constraints of existing computer technology permit only partial estimates of the degree of internal support of the *rbc*L sequence data for any *rbc*L phylogeny including the large number of species analyzed here. Bootstrap analysis with 200 bootstrap subsamples of the original data matrix and unordered characters (i.e., all base substitutions are equally weighted) was performed with local (i.e., "NNI" or "nearest neighbor interchange") swapping. A decay analysis was also performed (Bremer, 1988). A set of 18 trees of length 3117 was determined (specifying unordered characters). Trees one step longer (486 trees) and two steps longer (6237 trees) were also determined. (Note that an attempt to determine all trees up to length 3121 using PAUP 3.0s on a Macintosh Quadra 700 was aborted after 11.5 days of continuous execution. The analysis produced 14,873 trees occupying over 6 megabytes of memory and was estimated to be 10% complete.)

RESULTS

Our phylogenetic analyses of *rbc*L sequence data from monocotyledons show greater congruence with the taxonomic system of Dahlgren et al. (1985) than with other contemporary systems (reviewed in Goldberg, 1989). References to taxa in this report thus follow that system, with the superordinal endings modified to -anae, as suggested by Thorne (1992).

The topology with the greatest (least negative) maximum likelihood score (-18878.21) that was produced under the maximum likelihood method is given in Figure 1 as a best estimate of the *rbc*L phylogeny for the included species. Note that other topologies with likelihood scores that were not significantly different from that of the tree of Figure 1 were also produced (not shown). This tree can be organized into the following nine primary lineages diverging from an ancestor with an *rbc*L sequence most similar to the extant *Acorus calamus*. The taxonomic components (sensu Dahlgren et al., 1985) of each of these lineages in order of divergence from the root, are:

1) Alismatanae (4 spp.);
2) Aranae (4 spp.); (*Pleea tenuifolia* diverges after this clade and just prior to the remaining monocotyledons);
3) Velloziales (Bromelianae), Pandananae, and Cyclanthanae (1 spp. each);
4) Lilianae (7 of the 30 spp. analyzed);
5) Dioscoreales (2 of 3 spp. analyzed);
6) Lilianae (20 of 30 spp. analyzed) including all 16 species of Asparagales;
7) Arecanae (3 spp.);
8) Zingiberanae (11 spp.), Commelinaceae (3 spp.), and 1 species each of Pontederiales, Philydrales, and Haemodorales; and
9) Poales (4 spp.), Cyperales (5 spp.), Bromelianae (2 spp.), and Rapateaceae (1 spp.).

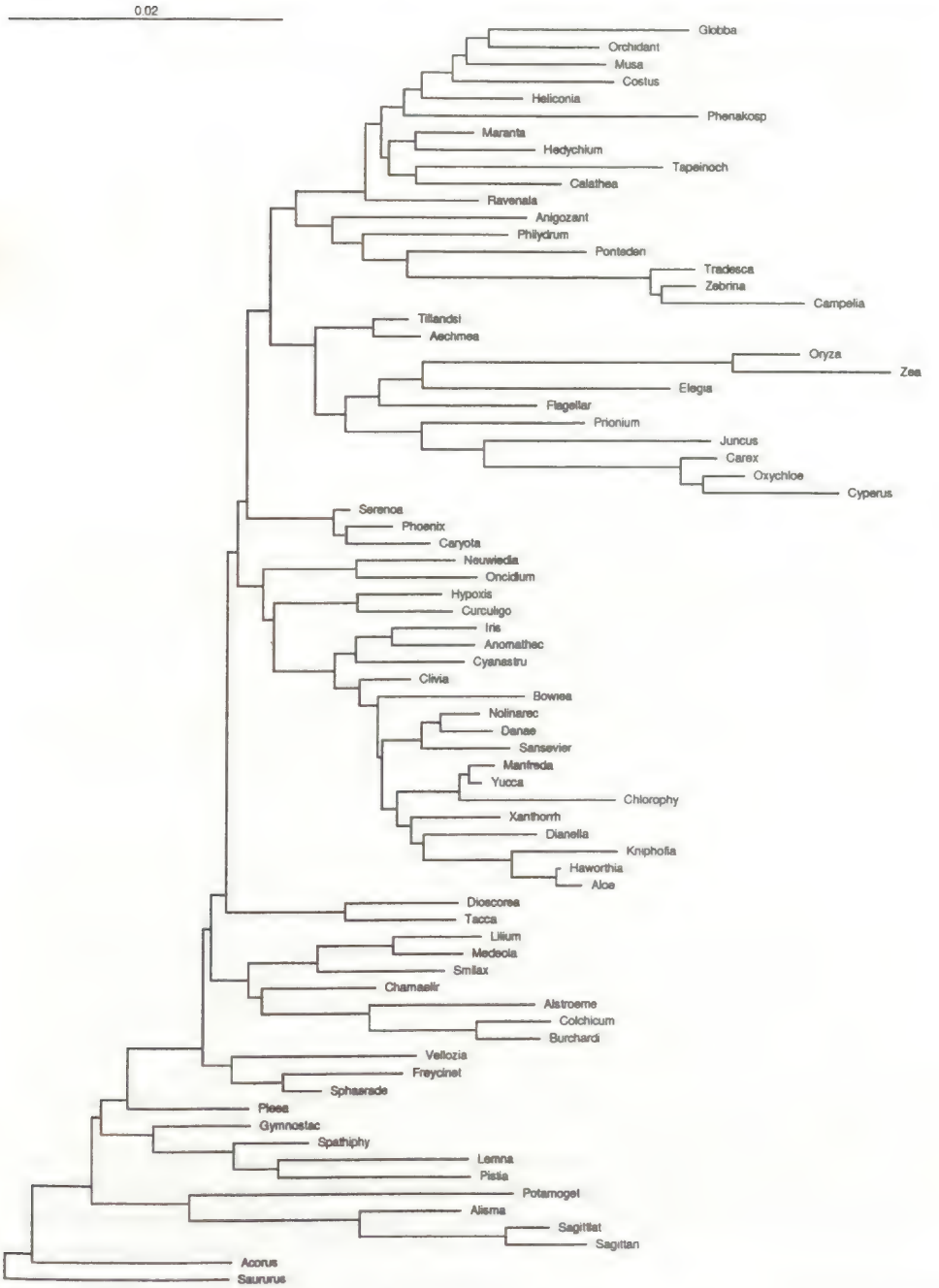


Figure 1. Maximum likelihood topology for 73 monocotyledons and the outgroup dicotyledon *Saururus cernuus* with the largest (least negative) log likelihood score. The analysis was executed using the computer program fastDNAml 1.0.3 (Olsen et al., 1992), on the Touchstone Delta Parallel Processing Supercomputer, the fastest existing computer. The initial phase of the analysis employed regional and local branch swapping with 33 replications of the input order and consumed more than 125 hours of supercomputer time. Additional rearrangements of the tree with the largest likelihood score produced the tree shown here. Scale bar indicates 0.02 maximum likelihood distance units.

Bootstrap results and decay index values for these species using PAUP are given in Figure 2. Note that because analysis of this large data set is computer intensive, only 200 bootstrap subsamples and local (NNI) branch swapping were employed, and trees up to only two steps longer were produced.

Parsimony analysis of *rbc*L sequences from 132 species with character state weighting produced 25 equally parsimonious trees (length with unordered characters: 4154; consistency index excluding uninformative characters: 0.258; retention index: 0.634). These trees differed from each other only by several rearrangements within each of Bromeliaceae and Arecaceae. An arbitrarily selected tree from this set is given in Figure 3 as an overview and in Figures 4 and 5 in detail. The maximum likelihood tree (Figure 1) and the set of parsimony trees (e.g., Figure 3) consist of the same major clades which diverge in the same order with only one exception: *Aletris farinosa* and three other Lilianae are found near the base of the clade dominated by Asparagales in the parsimony trees. Aside from this, only minor rearrangements are found, which may possibly be attributable to the additional species in the parsimony analysis.

DISCUSSION

The molecular trees resulting from the analysis of *rbc*L sequences from 500 species of seed plants (Chase et al., 1993) support many traditional phylogenetic concepts but also suggest relationships that contradict convention. The conifers are paraphyletic in this analysis, a condition that may merely reflect insufficient sampling (Chase et al., 1993). Three species of Gnetales form the immediate sister clade to the angiosperms, supporting a widely accepted hypothesis.

The basal clade among the angiosperms is occupied by the single, problematic, aquatic species, *Ceratophyllum demersum* (Les et al., 1991). The remaining angiosperms bifurcate into large clades, one characterized almost exclusively by species with monosulcate and monosulcate-derived types of pollen and the other consisting exclusively of species with tricolpate and tricolpate-derived pollen types (Chase et al., 1993).

Among the "triaperturate clade" of dicotyledons, the distribution of species of Saxifragaceae *sensu lato* serves as a preeminent example of *rbc*L sequence evidence that counters traditional systematics. The core members of Saxifragaceae *sensu lato*, i.e., the Saxifragoideae that were originally recognized on morphological, anatomical, and chemical grounds, are likewise well supported as a monophyletic group by the *rbc*L sequence data as well as by other molecular data (Downie et al., 1991; Morgan and Soltis, 1993). However, representatives of 16 of the 17 subfamilies of Saxifragaceae *sensu lato* were shown to be allied to at least 10 lineages in several subclasses of flowering plants (Morgan and Soltis, 1993). These relationships, determined by analysis of *rbc*L data, are supported by evidence from embryology, serology, and iridoid chemistry.

Phylogenetic analysis of *rbc*L sequences has also greatly improved our understanding of relationships among monocotyledons. In general, deep branches in the tree of Figure 1, such as those leading to one of the nine primary clades, are shorter than the terminal branches, indicating fewer nucleotide changes along the former. This relationship suggests either that the substitution rate during evolutionary radiations of the monocotyledons was unusually slow or that the original radiations occurred rapidly. The fossil record for angiosperms in general (Doyle and Hickey, 1976) and probably for the monocotyledons (Doyle, 1973), especially the record for fossil pollen, is consistent with the hypothesis of rapid radiation.

Measures of support are weak for the relative positions of the superorders of monocotyledons with one exception: Pandananae and Cyclanthanae are strongly supported as sister taxa (bootstrap value: 95%; decay value: two or more). The strong support of this

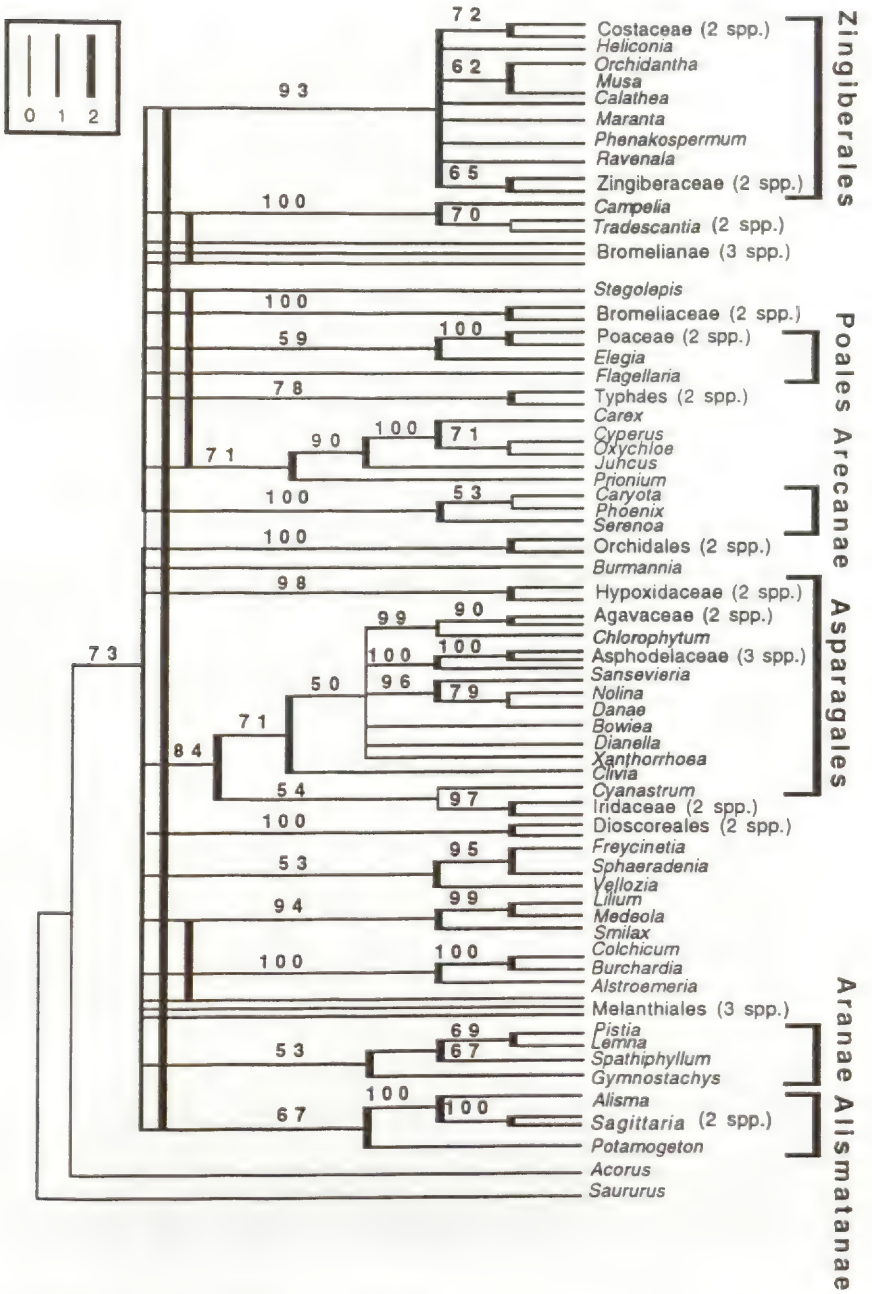


Figure 2. Majority rule (50%) consensus tree for *rbc*L data from monocotyledonous species. The bootstrap analysis was conducted using PAUP 3.0s with unordered characters and 200 bootstrap subsamples of the data matrix for the same species used to produce the maximum likelihood tree (Figure 1). Percentage values for those branches occurring in at least 50% of the bootstrap topologies are shown. Selected taxa are identified. Branch lengths are arbitrary. Decay values up to two steps longer are indicated as vertical bars of varying thickness overlaid on the bootstrap topology. The thickest lines indicate clades supported in trees at least two steps longer. The thinnest lines indicate clades supported only in maximum parsimony trees.

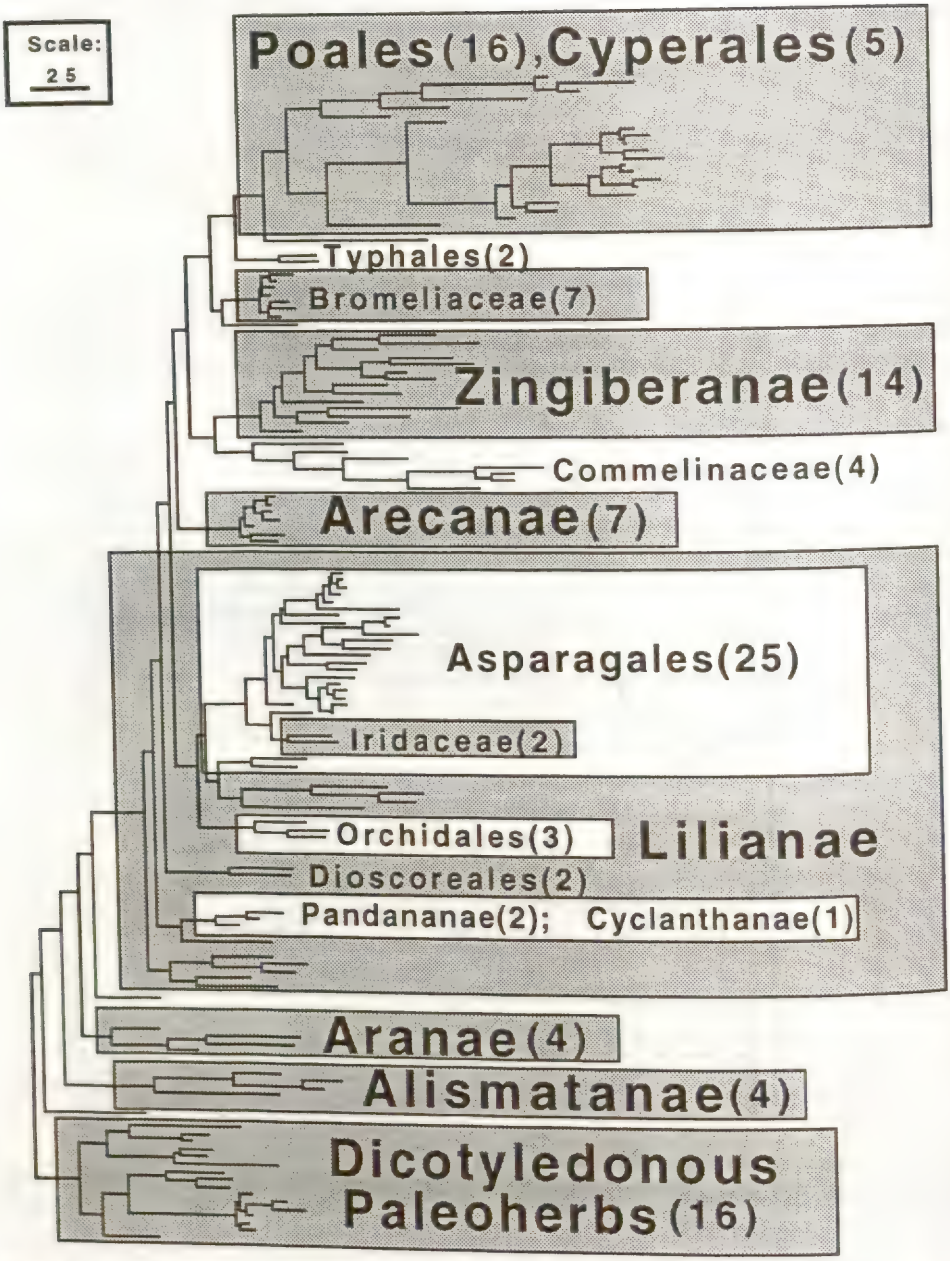


Figure 3. Topology of one, arbitrarily selected tree from a set of 25 equally parsimonious trees (of 4154 steps, consistency index of 0.258, and retention index of 0.634) for the *rbc*L nucleotide sequence data from 116 species of monocotyledons and 16 species of dicotyledons. The differences among the 25 trees were restricted to rearrangements within Bromeliaceae and Arecaceae. Branch lengths correspond to the number of inferred nucleotide substitutions optimized along the branches. The scale bar corresponds to 25 substitutions. The terminal species are identified in Figures 4 and 5 that together encompass this tree. Boxes, which may contain non-monophyletic clades, identify taxonomic components in the analysis. Number of species analyzed from each taxon are given parenthetically.

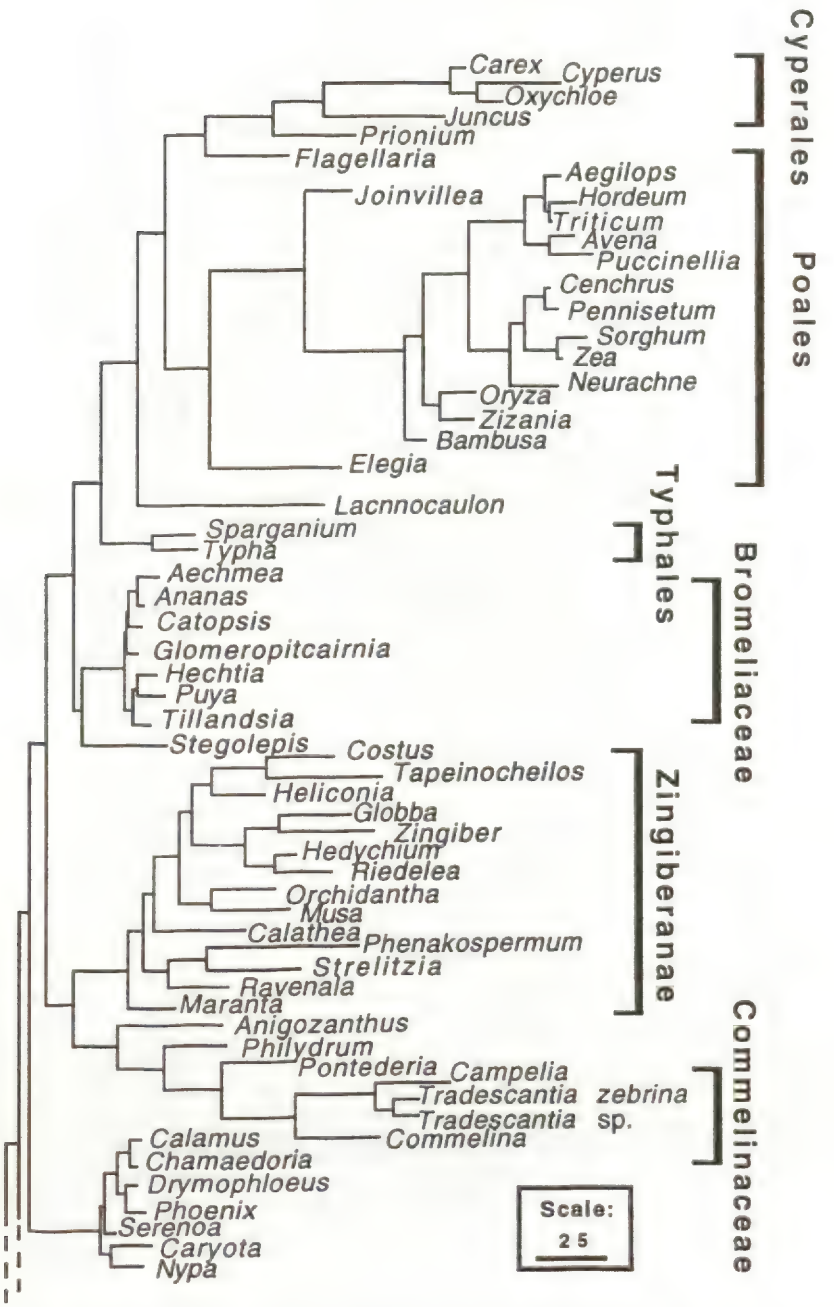


Figure 4. Terminal portion of the tree of Figure 3, including species of Commelinanae, Bromelianae, Zingiberanae, and Arecanae (see Figure 3 for positions of primary lineages in the complete tree). Branch lengths correspond to the number of substitutions optimized along the branches. The scale bar corresponds to 25 steps.

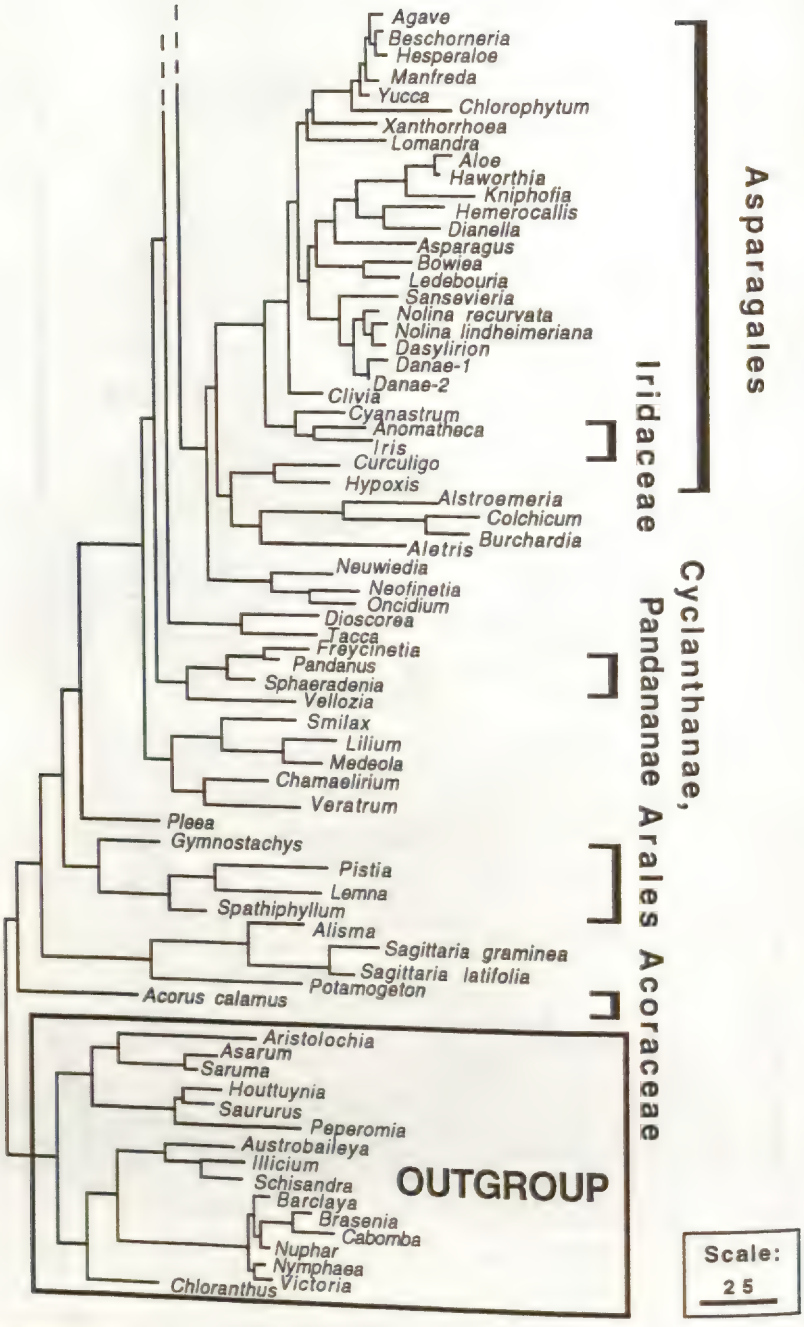


Figure 5. Basal portion of the tree of Figure 3, including species of Aranae, Alismatanae, Lilianae, and outgroup dicotyledons (see Figure 3 for positions of primary lineages in the complete tree). Branch lengths correspond to the number of substitutions along the branches. The scale bar corresponds to 25 steps. Note that *Acorus* occupies a singular, basal position relative to the other monocotyledons in this tree.

relationship is maintained when data from all available species of these superorders (*Freycinetia scandens* and *Pandanus veitchii* (Pandananae); *Ludovia integrifolia* and *Sphaeradenia pendula* (Cyclanthanae)) are analyzed (M. Duvall, analysis not shown). Those groups aside, the general lack of strong support at the highest taxonomic levels among the monocotyledons correlates with the generally decreased length of deeper branches in the trees.

The order of divergence of the major lineages in our parsimony analyses is in agreement with widely accepted views on the evolution of the monocotyledons. Recognition of the early divergence of Alismatanae (e.g., Cronquist, 1981) and the affinity between Alismatanae and Aranae (Dahlgren et al., 1985; Grayum, 1991) correlate with the position of species in these superorders near the base of the tree (Figures 1, 3, and 5). Later divergences of Arecanae (Doyle, 1973), Commelinanae, and Zingiberanae (Cronquist, 1981) are also consistent with our phylogenetic analysis.

Within the "uniaperturate clade," the monocotyledons are a monophyletic lineage. *Acorus calamus*, one of two species in that problematic genus, is sister to all other monocotyledons; this branch is supported by a bootstrap value of 73% and in trees at least two steps longer (Figure 2). Although *Acorus* traditionally has been included in Araceae because of morphological similarities with *Gymnostachys*, aroid authorities have recently revised classification of the family based on a substantial body of evidence (reviewed in Grayum, 1987) and proposed removal of *Acorus* to a monotypic family of uncertain affinities. An *rbc*L tree over the subset of 74 species constrained to include *Acorus* at the base of a monophyletic clade of four species of Arales (not shown) is five steps longer than the topology with *A. calamus* in a basal position. Our analysis strongly supports the revised classification that removes *Acorus* from Araceae and also offers an explanation for the failure to identify synapomorphies of this basal genus with other monocot groups.

Many facets of the *rbc*L trees presented here are discussed elsewhere (Duvall et al., 1993 and *Annals of the Missouri Botanical Garden* 80 (3)). Here we will focus on issues involving just three orders: Arales, Asparagales, and Poales. We have here included *rbc*L sequences from 25 species in 15 of 30 families of Asparagales sensu Dahlgren et al. (1985). Parsimony analysis (Figures 3 and 5) placed within one clade all of the included species of Asparagales plus six species of Lilianae, including Melanthiales (1 sp.) and Liliales (5 spp., including 2 of Iridaceae). The corresponding clade in the analysis of the smaller subset (Figure 2) is supported by bootstrap analysis at 84% and by decay analysis to at least two steps of decay. The *rbc*L data offer some support for the monophyly of this large order (estimated 5000 spp.), pending resolution of these relationships with Melanthiales and some Liliales.

The association suggested by phylogenetic analysis of *rbc*L sequence data of Iridaceae with Asparagales rather than with Liliales sensu Dahlgren et al. (1985) is not completely unexpected. Some of the diagnostic characteristics of Asparagales, such as woody growth habit (unknown in Liliales except for Iridaceae), septal nectaries, basifixed or hypopeltate anthers, loculicidal capsules, and few to one ovules per locule, are also to be found, at least occasionally, among Iridaceae, and particularly among basal genera (Goldblatt, pers. comm.). Familial characteristics for Iridaceae have sometimes been defined only for a few, relatively specialized genera (Goldblatt, 1987). These observations, together with our *rbc*L analyses, suggest possible affinities between Iridaceae and Asparagales.

Analyses of *rbc*L sequences of Poaceae have been reported previously for nine species (eight genera), using a dicotyledonous outgroup (Doebley et al., 1990). In our analysis of 13 species (13 genera), the pooid, panicoid, oryzoid, and bambusoid grasses are segregated into monophyletic clades (Figure 4), with the bambusoids (as *Bambusa multiplex*) forming a sister group to the rest of the family. This result suggests that infrafamilial relationships may be investigated for selected families using *rbc*L sequence data. The immediate sister clade to

Poaceae is *Joinvillea plicata* (Joinvilleaceae) and next divergent is a species of Restionaceae. This lineage of Poales is sister to a clade of Cyperales at the base of which is *Flagellaria indica* (Flagellariaceae; Poales). This topology is completely congruent with the distribution of three inversions in the plastid genomes of Commelinanae (Doyle et al., 1992).

The phylogenetic analysis presented here offers support for the recognition of nine primary lineages of monocotyledons. As noted, these trees are compatible with the taxonomic treatment of Dahlgren et al. (1985). However, the paraphyletic arrangements of Bromelianae, Commelinanae, and Lilianae in our analysis, perhaps a result of insufficient sampling among these taxa, warrant further study.

LITERATURE CITED

Albert, V., M. Chase, and B. Mishler. 1993. Character-state weighting for cladistic analysis of protein-coding DNA sequences. Ann. Missouri Bot. Gard. 80: 752–766.

_____, S. Williams, and M. Chase. 1992. Carnivorous plants: phylogeny and structural evolution. Science 257: 1491–1495.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

Chase, M., D. Soltis, R. Olmstead, D. Morgan, D. Les, B. Mishler, M. Duvall, R. Price, H. Hills, Y.-L. Qiu, K. Kron, J. Rettig, E. Conti, J. Palmer, J. Manhart, K. Sytsma, H. Michaels, W. J. Kress, K. Karol, W. D. Clark, M. Hedrén, B. Gaut, R. Jansen, K.-J. Kim, C. Wimpee, J. Smith, G. Furnier, S. Strauss, Q.-Y. Xiang, G. Plunkett, P. Soltis, S. Swensen, S. Williams, P. Gadek, C. Quinn, L. Eguiarte, E. Golenberg, G. Learn, Jr., S. Graham, S. Barrett, S. Dayanandan, and V. Albert. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528–580.

Clegg, M., K. Ritland, and G. Zurawski. 1986. Processes of chloroplast DNA evolution. Pp. 275–294 *in* S. Karlin and E. Nevo (eds.), Evolutionary Processes and Theory. Academic Press, New York.

Cronquist, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.

Dahlgren, R. 1983. General aspects of angiosperm evolution and macrosystematics. Nord. J. Bot. 3: 119–149.

_____, H. Clifford, and P. Yeo. 1985. The Families of the Monocotyledons. Springer-Verlag, New York.

Doebley, J., M. Durbin, E. Golenberg, M. Clegg, and D. Ma. 1990. Evolutionary analysis of the large subunit of carboxylase (*rbc*L) nucleotide sequence among the grasses (Gramineae). Evolution 44: 1097–1108.

Donoghue, M. J. and J. Doyle. 1989. Phylogenetic studies of seed plants and angiosperms based on morphological characters. Pp. 181–193 *in* R. Fernholm, K. Bremer, and H. Jornvall (eds.), The Hierarchy of Life. Elsevier Science Publishers, Amsterdam.

_____, R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Ann. Missouri Bot. Gard. 79: 333–345.

Downie, S., R. Olmstead, G. Zurawski, D. Soltis, P. Soltis, J. Watson, and J. Palmer. 1991. Loss of the chloroplast DNA *rbl*2 intron demarcates six lineages of dicotyledons: molecular and phylogenetic implications. Evolution 45: 1245–1259.

Doyle, J. A. 1973. The monocotyledons: their evolution and comparative biology. Quart. Rev. Biol. 48: 399–413.

_____ and L. Hickey. 1976. Pollen and leaves from the mid-Cretaceous Potomac Group and their bearing on early angiosperm evolution. Pp. 139–206 *in* C. Beck (ed.), Origin and Early Evolution of Angiosperms. Columbia University Press, New York.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

_____, J. I. Davis, R. J. Soreng, D. Garvin, and M. J. Anderson. 1992. Chloroplast DNA inversions and the origin of the grass family (Poaceae). Proc. Natl. Acad. Sci. U.S.A. 89: 7722–7726.

Duvall, M., M. Clegg, M. Chase, W. D. Clark, W. J. Kress, H. Hills, L. Eguiarte, J. Smith, B. Gaut, E. Zimmer, and G. Learn, Jr. 1993. Phylogenetic hypotheses for the monocotyledons constructed from *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 607–619.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Molec. Evol. 17: 368–376.

Gaut, B., S. Muse, W. D. Clark, and M. Clegg. 1992. Relative rates of nucleotide substitution at the *rbc*L locus of monocotyledonous plants. J. Molec. Evol. 35: 292–303.

Giannasi, D., G. Zurawski, G. Learn, Jr., and M. Clegg. 1992. Evolutionary relationships of the Caryophyllidae based on comparative *rbc*L sequences. Syst. Bot. 17: 1–15.

Goldberg, A. 1989. Classification, evolution, and phylogeny of the families of monocotyledons. Smithsonian Contr. Bot. 71: 1–73.

Goldblatt, P., P. Rudall, V. Cheadle, L. Dorr, and C. Williams. 1987. Affinities of the Madagascan endemic *Geosiris*, Iridaceae or Geosiridaceae. Bull. Mus. Natl. Hist. Nat., B, Adansonia 9: 239–247.

Golenberg, E., D. Giannasi, M. Clegg, C. Smiley, M. Durbin, D. Henderson, and G. Zurawski. 1990. Chloroplast DNA sequence from a Miocene *Magnolia* species. Nature 344: 656–658.

Grayum, M. 1987. A summary of evidence and arguments supporting the removal of *Acorus* from the Araceae. Taxon 36: 723–729.

_____. 1991. Systematic Embryology of the Araceae. Bot. Rev. 57: 167–203.

Jarrell, D., M. Roose, S. Traugh, and R. Kupper. 1992. A genetic map of citrus based on segregation of isozymes and RFLPs in an intergeneric cross. Theor. Appl. Genet. 84: 49–56.

Kim, K.-J., R. K. Jansen, R. S. Wallace, H. J. Michaels, and J. D. Palmer. 1992. Phylogenetic implications of *rbc*L sequence variation in the Asteraceae. Ann. Missouri Bot. Gard. 79: 428–445.

Les, D. H., D. Garvin, and C. Wimpee. 1991. Molecular evolutionary history of ancient aquatic angiosperms. Proc. Natl. Acad. Sci. U.S.A. 88: 10119–10123.

Martin, P. G. and J. M. Dowd. 1989. Phylogeny among the flowering plants as derived from amino acid sequence data. Pp. 195–204 *in* B. Fernholm, K. Bremer, and H. Jornvall (eds.), The Hierarchy of Life. Elsevier Press, Amsterdam.

Morgan, D. R. and D. E. Soltis. 1993. Phylogenetic relationships among members of Saxifragaceae *sensu lato* based on *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 631–660.

Olmstead, R. G., H. J. Michaels, K. M. Scott, and J. D. Palmer. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbc*L. Ann. Missouri Bot. Gard. 79: 249–265.

Olsen, G., H. Natauda, R. Hagstrom, and R. Overbeek. 1992. FastDNAml Version 1.0.3. University of Illinois, Urbana, and Argonne National Laboratory, Argonne, Illinois.

Palmer, J. 1986. Isolation and structural analysis of chloroplast DNA. Meth. Enzymology 118: 167–186.

Ritland, K. and M. Clegg. 1987. Evolutionary analysis of plant DNA sequences. Amer. Naturalist 130, supplement: S74–S100.

Soltis, P. S., D. E. Soltis, and C. J. Smiley. 1992. An *rbc*L sequence from a Miocene *Taxodium* (bald cypress). Proc. Natl. Acad. Sci. U.S.A. 89: 449–451.

Swofford, D. 1990. PAUP: phylogenetic analysis using parsimony, version 3.0m. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Takhtajan, A. L. 1980. Outline of the classification of flowering plants (Magnoliophyta). Bot. Rev. 46: 225–359.

Thorne, R. F. 1981. Phytochemistry and angiosperm phylogeny: a summary statement. Pp. 233–295 *in* D. Young and D. Seigler (eds.), Phytochemistry and Angiosperm Phylogeny. Praeger, New York.

_____. 1992. Classification and geography of the flowering plants. Bot. Rev. 58: 225–348.

Wilson, M., B. Gaut, and M. Clegg. 1990. Chloroplast DNA evolves slowly in the palm family (Arecaceae). Molec. Biol. Evol. 7: 303–314.

PATTERNS OF TRANSPOSABLE ELEMENT EVOLUTION IN THE GRASSES[1]

Amy F. MacRae[2]

ABSTRACT. The existence and unique properties of plant transposable elements can have several effects on systematic inference. Selected features of transposable elements are examined and discussed with relation to systematic inference. These features include: high levels of size polymorphism within transposon groups, the multiple-copy nature of transposons, horizontal transmission of transposons, and species-specific patterns of transposon distribution in the genome. In addition, I raise the question "What systematic inferences can be made about transposons themselves, apart from any use they may have as markers of organismal phylogeny?" A case history in point is the evolution of the *Ac*/*Ds* family of transposable elements in maize and the related grass pearl millet. An *Ac*-like element was cloned from a DNA library of the grass pearl millet (*Pennisetum glaucum*) and 2387 bp of it have been sequenced. The pearl millet *Ac* sequence was then aligned with the corresponding *Ac* region from maize. Sequence comparisons and other data support the theory that *Ac*-like sequences were probably present in the ancestor of maize and pearl millet, and have thus existed in the grasses for at least 25 million years. Because *Ac*-like sequences have an ancient origin, such sequences may also exist in many widely divergent grasses, in addition to pearl millet.

INTRODUCTION

Transposable elements are defined as pieces of mobile DNA. They have been found in virtually every organism in which they have been sought (Fedoroff, 1989a, 1989b). Transposable elements differ from simple, Mendelian genes in that they can move in the genome, excising from one chromosomal location and inserting into another. This paper will focus on plant transposable elements, addressing the following three topics: I. The unique properties and dynamics of plant transposable elements; II. The use of plant transposable elements for systematic inferences; III. A case history of transposable element evolution in the grasses.

I. THE UNIQUE PROPERTIES AND DYNAMICS OF PLANT TRANSPOSABLE ELEMENTS

Transposable elements were first documented when the "non-autonomous" element named *Ds* (*Dissociation*) was shown by McClintock to move from one location on the short arm of a chromosome 9 homologue, to a more distal location on the same homologue in *Zea mays* (McClintock, 1945, 1946, 1948, 1950a, 1950b, 1951a, 1951b). Although transposable elements have now been found in many other plants (see review by Nevers et al., 1986), maize transposable elements are the most well characterized at the molecular level (Fedoroff, 1989b). Therefore, the examples given here will be from maize transposable element systems.

[1]This work was supported in part by postdoctoral grant #88-3-10ME from the Alfred P. Sloan Foundation, and by NSF grant BSR-8500206 to Michael T. Clegg. I appreciate critical comments from Gary Brown, Michael Clegg, and an anonymous reviewer. I also appreciate computer and laboratory assistance from Jerry Learn and Merja Clegg, respectively.

[2]Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Rd., St. Louis, Missouri 63121, U.S.A.

Maize transposable elements have several properties and dynamics that make them different from other nuclear genes. For example, most maize transposable elements occur as two-element families, one member being "autonomous" and the other member being "non-autonomous" (McClintock, 1950a). So-called "autonomous" elements can catalyze their own transposition, as well as the transposition of "non-autonomous" elements. "Non-autonomous" elements can only transpose when an "autonomous" element is also present in the genome. In maize, examples of "autonomous" transposable elements include *Activator* (*Ac*) and *Suppressor-mutator* (*Spm*) (Fedoroff, 1989b). The "autonomous" elements *Ac* and *Spm* each have a "non-autonomous" complement, called *Dissociation* (*Ds*) and *defective Suppressor-mutator* (*dSpm*), respectively (Fedoroff, 1989b). In the case of the maize transposable element called *Mutator* (*Mu*), nine different classes of elements have been isolated to date, all of which are thought to be "non-autonomous" (see references in Chomet et al., 1991). In addition, "autonomous" or regulator elements of the *Mu* family have also recently been isolated (Chomet et al., 1991; Qin and Ellingboe, 1990).

Some of the most interesting general features of maize transposable elements are exemplified by the *Activator/Dissociation* (*Ac/Ds*) transposon family. Four genetic properties of *Ac/Ds* are shown in Figure 1, reproduced from Fedoroff (1989b). As illustrated in Figure 1a, certain *Ds* elements have the ability to break chromosomes, in the presence of *Ac*. We now know transposons can also mediate other macromutations, such as deletions, inversions, duplications, and translocations of adjacent, non-element DNA (Charlesworth, 1985; Fedoroff, 1989b; McClintock, 1951a, 1978; Sherratt et al., 1983). Figure 1b shows the hallmark of transposable elements: their ability to transpose or move from one chromosomal site to another. Figure 1c depicts transposon mutation. Through mutations that delete internal *Ac* regions, an *Ac* element can become an element with the genetic properties of *Ds*. Thus, many *Ds* elements are called "deletion-derivatives" of *Ac*: they have become permanently mutated into the "non-autonomous" form. Figure 1d depicts reversible inactivation of *Ac*: it can go from an active to an inactive state, and *vice versa*. In other words, when inactive, *Ac* elements behave genetically like *Ds* elements, but they differ from actual *Ds* elements because they have the ability to return to the active *Ac* state. This temporary inactivation of plant transposons is often associated with element DNA methylation (Fedoroff, 1989b).

One unique feature of plant transposable elements is their high level of size polymorphism. Within a given transposon family, there can exist large differences in total transposon length, as well as vast differences in internal sequence content. As seen in Figure 2 (from Fedoroff, 1989b), the maize *Activator* (*Ac*) element has many related *Dissociation* (*Ds*) elements that are deletion-derivatives of it. Such *Ds* elements, including *Ds9*, *Ds2d1*, *Ds2d2*, and *Ds6*, are pictured below *Ac* (Figure 2). These *Ds* elements are identical with *Ac* in the regions depicted by the solid lines, but differ from *Ac* by internal deletions, represented by the parentheses enclosing blank areas. Thus, maize *Ds* elements as a group vary widely in total size as well as in internal sequence content.

A second example of transposon polymorphism is given in Figure 3, taken from Fedoroff (1989b). Like *Ds* elements, many of which are deletion-derivatives of *Ac*, *dSpm* elements are also frequently deletion-derivatives of *Spm*. Figure 3 shows the *dSpm-7995*, *dSpm-7997B*, *dSpm-8004*, *dSpm-8*, and *Spm-w-8011* alleles, all of which have internal deletions relative to the standard 8.3 kb *Spm* element (Fedoroff, 1989b). Thus, *dSpm* elements as a group can also vary widely in total size as well as in internal sequence content. It should be noted that Figure 3 serves primarily to highlight the size differences among *dSpm* elements. For an accurate account of maize *Spm* translational regulation involving differential RNA splicing, one is referred to Masson et al. (1989).

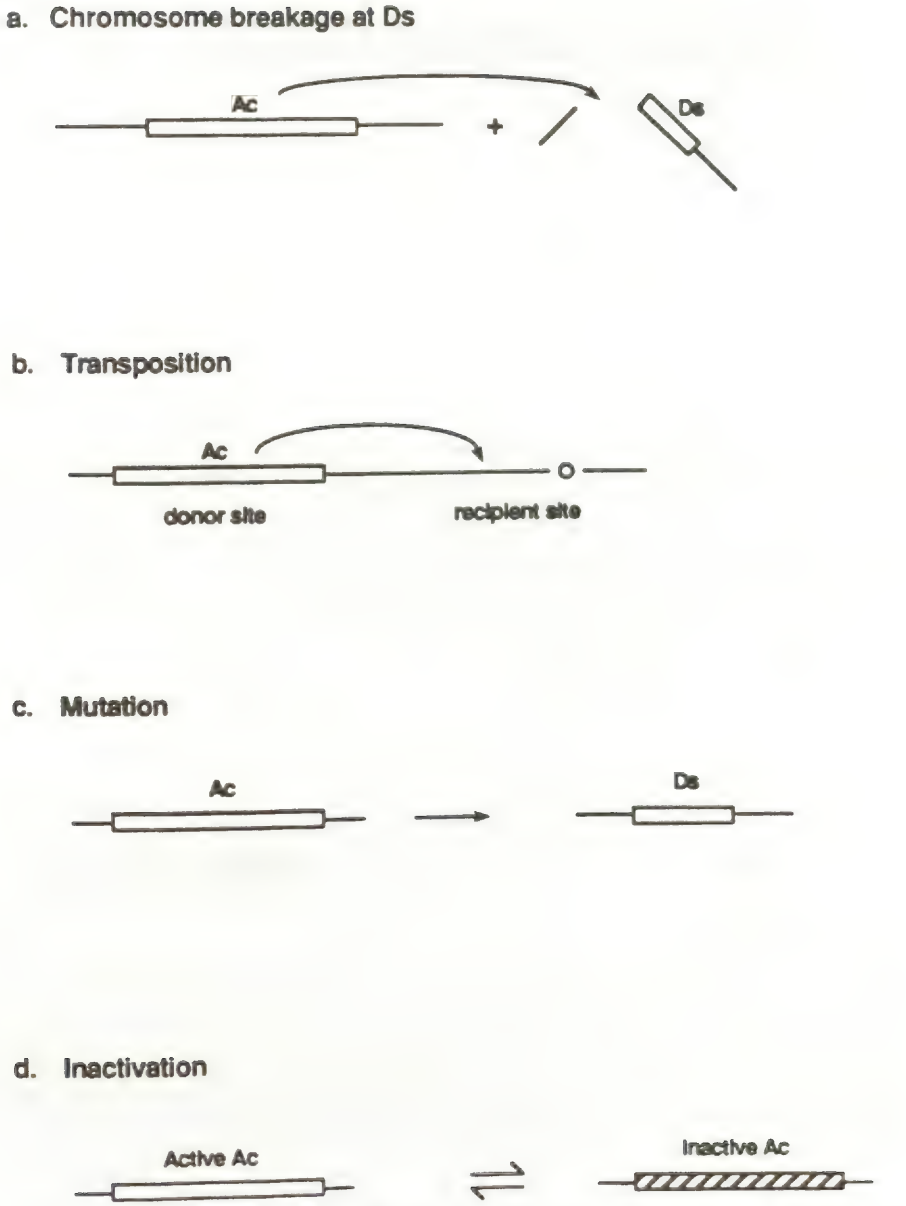


Figure 1. Four genetic properties characteristic of the maize *Ac* / *Ds* transposable element family. Reproduced with permission from Fedoroff (1989b). **a.** Certain *Ds* elements have the ability to cause chromosome breakage, in the presence of *Ac*. **b.** The *Ac* element is shown moving or transposing from a donor site to a recipient site on the chromosome. **c.** Transposable elements can undergo mutation. Here an *Ac* element has been transformed into an element with the genetic properties of *Ds*, through internal deletions in *Ac*. **d.** *Ac* can be reversibly inactivated. This temporary inactivation is often associated with element methylation.

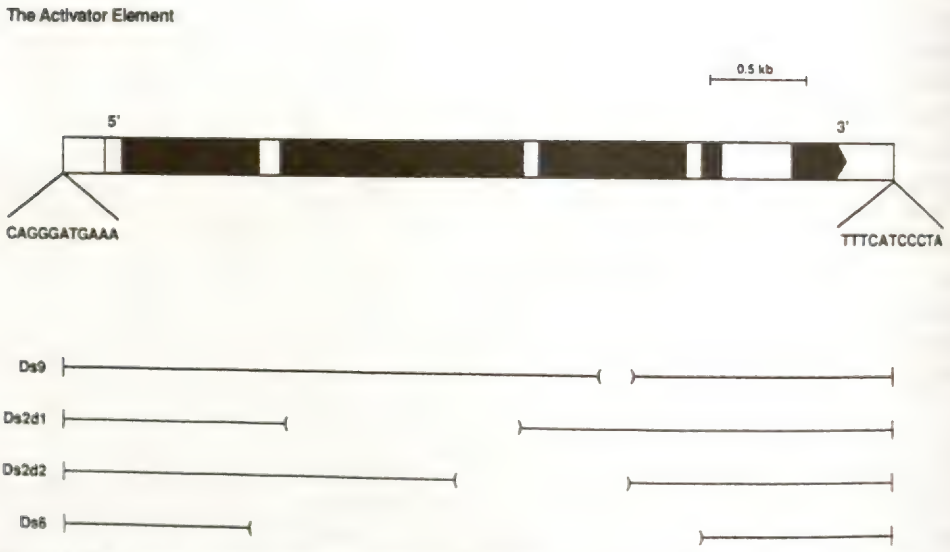


Figure 2. The maize *Activator* (*Ac*) element and several *Ds* "deletion-derivatives" of *Ac*. Reproduced with permission from Fedoroff (1989b). The maize *Activator* element is shown on top. The five exons are in black and four introns are in white. The sequences of the 11 bp *Ac* terminal inverted repeats are given. Several *Ds* elements are also shown. Sequences in which the *Ds* elements are identical with *Ac* are represented by solid horizontal lines; sequences differing from *Ac* by internal deletions are represented by blank areas bordered by parentheses. This figure highlights the large size polymorphism that exists within *Ds* elements. *Ds9*, *Ds2d1*, *Ds2d2*, and *Ds6* are all *Ds* elements, but they have different sizes and different internally deleted regions.

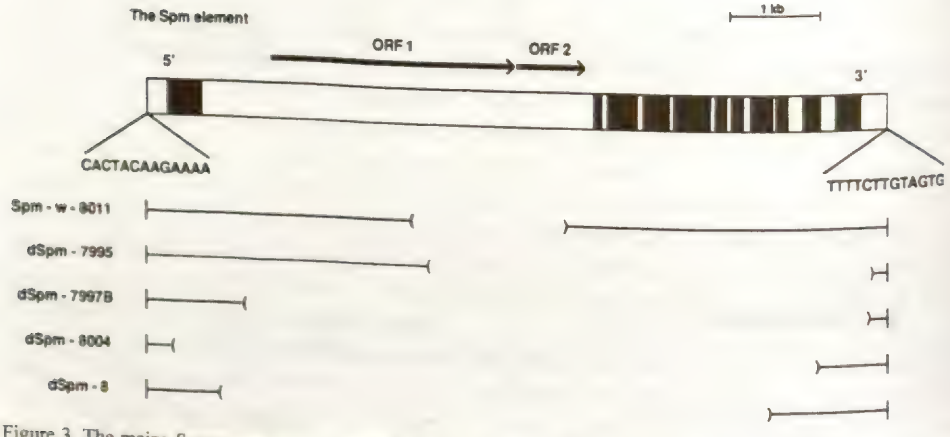


Figure 3. The maize *Suppressor-mutator* (*Spm*) element and several *dSpm* "deletion derivatives." Reproduced with permission from Fedoroff (1989b). The maize *Suppressor-mutator* (*Spm*) element is represented on top. The 5′ and 3′ ends of the transcription unit are shown. The eleven black areas within the transcription unit are exons; the ten white areas within the transcription unit are introns. Below the *Spm* element are several *dSpm* elements that are "deletion derivatives" of *Spm*. The blank areas bordered by parentheses indicate those internal regions that are missing from various *dSpm* elements relative to the standard *Spm* element shown on top. This figure highlights the large size and internal sequence polymorphism within *dSpm* elements.

A final example of transposon polymorphism is given in Figure 4, taken from Fedoroff (1989b). This figure depicts two transposition-defective members of the *Mutator* element family, *Mu1* and *Mu1.7*. *Mu1* is 1.4 kb in length and has 0.2 kb inverted repeat (IR) sequences at its ends. Inverted repeats are depicted by black, filled arrows. The *Mu1.7* element is 1.75 kb in length, also has 0.2 kb inverted repeat sequences depicted by black filled arrows, but it has an internal insertion of 385 bp relative to the *Mu1* element. This demonstrates the presence of internal sequence and size polymorphism in *Mutator* elements. In fact, nine different classes of *Mutator* elements have been isolated, and their ~220 bp inverted repeats all share sequence similarity (see Chomet et al., 1991). However, different *Mu* element classes have internal sequences that are completely unrelated to one another (Chomet et al., 1991). This further demonstrates the large polymorphism and lack of sequence-relatedness that can exist within a single family of transposons.

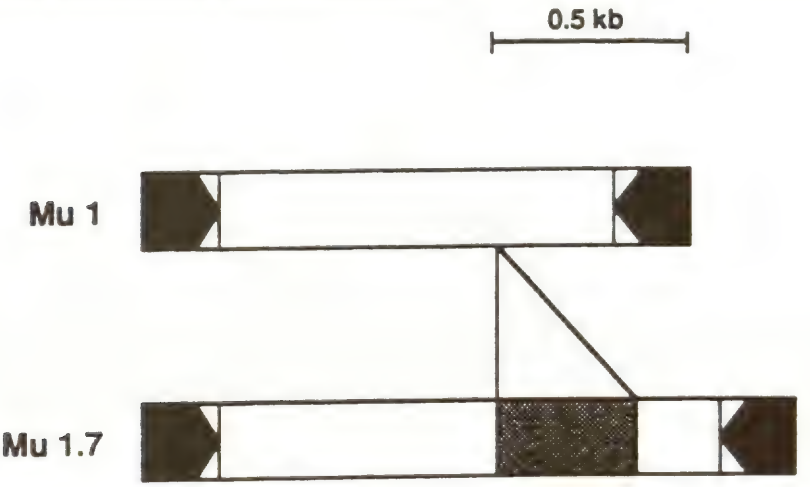


Figure 4. The maize *Mu1* and *Mu1.7 Mutator* elements. Reproduced with permission from Fedoroff (1989b). Depicted here are two transposition-defective members of the *Mutator* element family. *Mu1* is 1.4 kb in length and has 0.2 kb inverted repeats at its ends, shown by black inverted filled arrows. *Mu1.7* is 1.75 kb in length and also has 0.2 kb inverted repeats at its ends, but has an internal insertion of 385 bp (shown in grey) relative to *Mu1*. This demonstrates the presence of internal sequence and size polymorphism within maize *Mutator* elements. See text for additional examples of *Mutator* internal sequence polymorphism.

In addition to size polymorphism, another notable feature of plant transposable elements is their multiple copy nature. In maize, the *Activator* (*Ac*) element has 4–10 copies per genome (Chomet et al., 1987; Fedoroff et al., 1983), *Dissociation* (*Ds*) elements typically have 30–100 copies per genome (Freeling, 1984; Vershinin et al., 1987; Wessler, 1988), *Mutator* (*Mu*) elements can have between 10 and 100 copies per genome (Fedoroff, 1989a), and *defective Spm* (*dSpm*) elements can have 30–100 copies per genome.

A third feature of transposable elements is their ability to be transmitted horizontally. Horizontal transmission is defined as the transfer of genetic information between the genomes of two species (Li and Graur, 1991). This process differs from normal vertical transmission of genetic information since vertical transmission involves sexual reproduction and horizontal transmission does not. There are two requirements for the horizontal transfer of genetic information: (1) a vector to transfer the genetic material between organisms and cells; (2) a molecular mechanism to insert the transferred DNA into the host organism's genome (Li and Graur, 1991). Potential transfer vectors include retroviruses and plasmids (Bishop, 1981; Li and Graur, 1991; Varmus, 1983).

One proposed example of transposon horizontal transmission is particularly well documented in *Drosophila* by Houck et al. (1991). They propose that the *P* transposable element has been transferred from the *Drosophila willistoni* species group to a member of the *Drosophila melanogaster* species group, via a semiparasitic mite vector, *Proctolaelaps regalis* (Figure 5). Houck et al. (1991) offer the following evidence in support of their theory: (1) *P* element sequences taken from the two *Drosophila* species in question are nearly identical. (2) There is a lack of congruence between *P* element sequence identity and the divergence time between the two *Drosophila* species pairs. (3) Samples of the mite associated with a *P* element strain of *D. melanogaster* have been shown to carry *P* element sequences, as well as *Drosophila* ribosomal DNA sequences. (4) *Proctolaelaps regalis* feeds on *Drosophila* eggs and larvae. (5) The two *Drosophila* species in question have overlapping geographical ranges in certain regions. It is these types of exact criteria that are needed to adequately document transposon horizontal transmission.

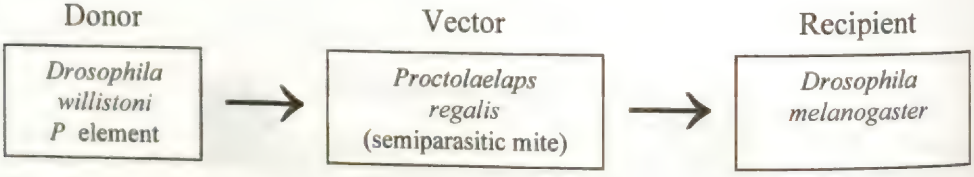


Figure 5. Proposed horizontal transfer of the *P* element from one *Drosophila* species group to another *Drosophila* species group, via a mite vector. Diagram adapted from an article by Houck et al. (1991). The proposed donor of the *P* element is a member of the *Drosophila willistoni* species group, the recipient is a member of the *D. melanogaster* species group, and the proposed vector for horizontal transmission is a semiparasitic mite, *Proctolaelaps regalis*. The mite feeds on *Drosophila* eggs and larvae and was found to contain *P* element sequences. Several lines of evidence are presented (see text) which substantiate the theory of a recent horizontal transfer of the *P* element into *Drosophila melanogaster* via such a mechanism.

II. THE USE OF PLANT TRANSPOSABLE ELEMENTS FOR SYSTEMATIC INFERENCES

Extensive size polymorphism within a transposon family would hinder the use of transposons as probes for inferring systematic relatedness. Both large size differences and internal deletions or internal polymorphism (Figures 2, 3, and 4) would make it difficult to perform large-scale sequence alignments between transposable elements from different organismal taxa.

Systematic inference using transposons would also be hindered by the multiple copy nature of most transposon families. Multiple copies of any sequence pose two main problems for systematic inference. The first problem is that of paralogous comparisons between copies. Paralogous comparisons involve two different forms of a sequence derived from a transposon duplication event rather than from an organismal speciation event. In Figure 6 (from Li and Graur, 1991), two sequences α and β (assume they are members of a transposon family) derive from an ancestral duplication event. Then speciation occurs. If we randomly sample and compare transposon copy α from species 1 and transposon copy β from species 2 in the present time, we would be making a paralogous comparison. We would be comparing two sequences that began diverging at the time of gene duplication (T_D time units ago), and not at the time of speciation (T_S time units ago; see Figure 6). It is often difficult to know what type of comparison is being made: paralogous (comparing genes α_1 and β_2) or orthologous (comparing genes α_1 and α_2 or β_1 and β_2; Figure 6).

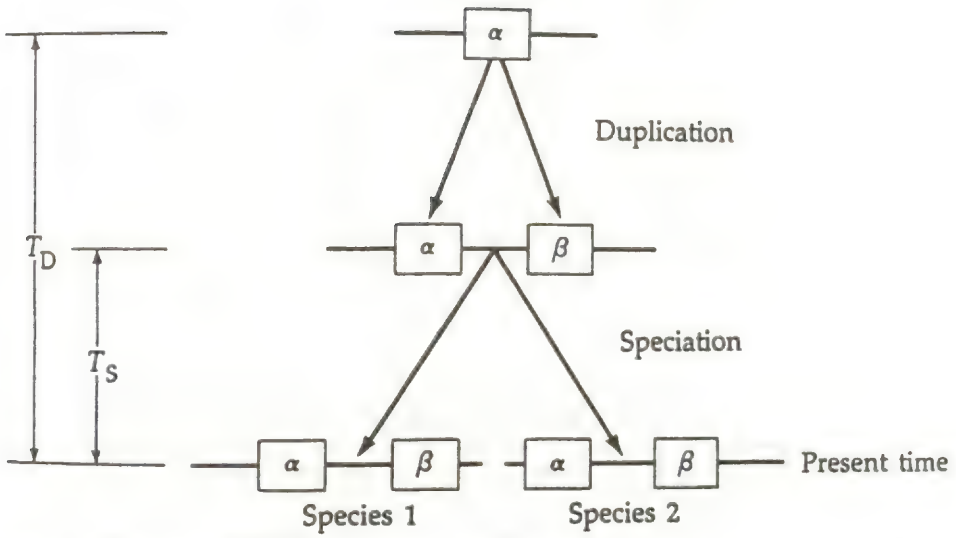


Figure 6. Diagram of gene duplication, organismal speciation, and the difference between paralogous versus orthologous comparisons among sequence copies. Reproduced with permission from Li and Graur (1991). Two sequences α and β (assume they are transposons) derive from an ancestral duplication event, T_D time units ago. Speciation then occurs to produce two species 1 and 2, T_S time units ago. If we sample and compare two sequences in the present time, the α sequences in species 1 and 2 are orthologous, as are the β sequences. However, the α sequences are paralogous to the β sequences.

The second potential problem with multiple copy sequences is that of concerted evolution between copies. This is a phenomenon where individual copies do not evolve independently, but instead evolve in concert due to genetic interactions among gene family members (Arnheim, 1983; Dover, 1982; Ohta, 1980). Due to the process of concerted evolution, members of a repeated-sequence family can be very similar within a species, but can differ greatly between even closely related species (Brown et al., 1972).

Concerted evolution has been observed in several multigene families, including ubiquitin genes and the NTS region of ribosomal genes (Arnheim, 1983; Brown et al., 1972; Dover, 1982; Ohta, 1980; Sharp and Li, 1987; Zimmer et al., 1980). The mechanisms responsible for concerted evolution include unequal crossing over, gene conversion, transposition, and replication slippage (Li and Graur, 1991). All of these processes could also potentially affect multi-copy transposable elements. For example, unequal crossing over within an intron of the low-density lipoprotein receptor gene is thought to have occurred due to the alignment of multiple *Alu* sequences within the introns of this gene (Hobbs et al., 1986). The processes of concerted evolution would thus hinder our ability to make correct systematic inferences using multi-copy markers of phylogeny, such as transposons.

The third feature of transposable elements that would affect their use in systematic inference is horizontal transmission (see Figure 7, taken from Li and Graur, 1991). If a transposable element were used as a marker to infer phylogenetic relationships among three species A, B, and C, and if that element had been transferred horizontally from species B to species C during evolution, one would infer an incorrect phylogenetic tree (Figure 7).

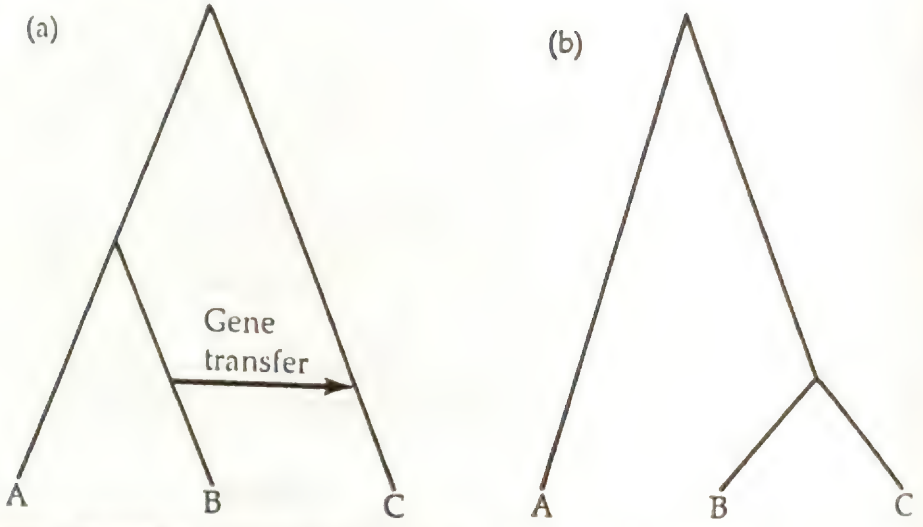


Figure 7. Phylogenetic tree reconstruction in the case of horizontal transfer of a transposon. Reproduced with permission from Li and Graur (1991). If a transposable element were used as a marker to infer phylogenetic relationships among three species A, B, and C, and if that element had been transferred horizontally from species B to species C during evolution, one would infer an incorrect phylogenetic tree (b) rather than the true tree (a).

Despite its potentially large impact on systematic inference, the phenomenon of horizontal transmission probably occurs at low frequency in transposons with short inverted repeats (Kidwell, 1992). "Short inverted repeat" (IR) transposons are the class of transposons discussed thus far in this paper: *Ac*, *Spm*, *Mu*, and *P* elements. They all have short inverted repeats (IRs) at their terminii, and they use a DNA to DNA transposition mechanism. Cases of horizontal transmission, like the one described here (Houck et al., 1991) involving the *P* element (Figure 5), are probably rare. The reason for this is that several stringent criteria must be met for horizontal transfer to be observed (Kidwell, 1992): (1) there must be DNA contact between donor and recipient organisms, mediated by a vector; (2) donor DNA must integrate into the recipient's germ line; (3) newly integrated DNA must become represented at least one time in all individuals of a population (i.e., must become fixed and not lost); (4) the lineage carrying the transferred DNA must be sampled.

Because of these stringent requirements, horizontal transmission is probably not observed at high frequency among short IR transposons. It has been observed at high evolutionary frequencies, however, in two groups of retroelements, i.e., elements that transpose using reverse transcription of an RNA intermediate: the *Ty1-copia* group (Flavell et al., 1992) and the *Ty3-gypsy* group (Doolittle et al., 1989; Voytas, 1992). Retroelements, which have a higher observed frequency of horizontal transmission than do short inverted repeat transposons, would therefore probably produce greater systematic incongruities than would short inverted repeat transposons, were either used as probes in systematic studies.

As we have seen, the combined features of transposons (the large amount of within-family size and deletion polymorphism, multiple copies, and the potential for horizontal transmission) could all have negative effects on systematic inference. However, before we conclude that transposons are worthless in phylogeny reconstruction, let us assume a broader perspective. If we consider not just single transposon copies, but look instead at total copy number and transposon genomic distribution on all chromosomes, transposons might actually be useful as species-specific markers. In fact, the distribution and amount of repetitive DNA often shows a high degree of species specificity (Dover, 1982), in both prokaryotes and eukaryotes. For example, *E. coli* and *Salmonella typhimurium* have very similar chromosome

maps (Riley and Krawiec, 1987), but differ greatly in their distribution of IS elements (Shapiro, 1992). In eukaryotes, *Drosophila simulans* and *D. melanogaster* have very similar genetic maps and chromosome banding patterns, but have very different distributions of repeated DNA (Dowsett, 1983; Shapiro, 1992). Thus, whole genomic patterns of transposon distribution may be far more useful taxonomic characters in reconstructing organismal phylogenies than are individual transposon DNA sequences or restriction maps.

III. A CASE HISTORY OF TRANSPOSABLE ELEMENT EVOLUTION IN THE GRASSES

The evolution of plant transposable elements is of great interest in itself, apart from any use transposons might have as markers of organismal phylogeny. With this in mind, we wish to ask two questions: (1) What is the evolutionary history of plant transposons? and (2) How do plant transposons evolve at the DNA sequence level? These two questions will be addressed within the *Ac*/*Ds* transposable element system of maize and related grasses, because this is the transposon family about which we have the most molecular and evolutionary information.

We have chosen to study the *Ac* transposable element in two very divergent grasses: maize and pearl millet. The *Ac* element system was chosen for several reasons. It was the first transposon family described by Barbara McClintock (McClintock, 1948, 1951a). In addition, *Ac* is small, with well-characterized molecular biology (Fedoroff, 1989b), and has been completely sequenced in maize (Behrens et al., 1984; Chen et al., 1987; Fedoroff et al., 1983; Fedoroff et al., 1984; Kunze et al., 1987; Muller-Neumann et al., 1984; Pohlman et al., 1984).

The completely sequenced maize *Ac* element is 4.6 kb in length, has 11 bp imperfect terminal inverted repeats, and generates an 8 bp duplication of target site genomic DNA upon insertion (Behrens et al., 1984; Chen et al., 1987; Fedoroff et al., 1983, 1984; Kunze et al., 1987; Muller-Neumann et al., 1984; Pohlman et al., 1984). The element has 5 exons and 4 introns (see Figures 2 and 8), and encodes a single 3.5 kb mRNA. The processed mRNA contains a 2421-nucleotide open reading frame (ORF), encoding a protein of 807 amino acids (Kunze et al., 1987). The 2421-nucleotide ORF is thought to encode the "transposase" or enzyme required for transposition (Kunze and Starlinger, 1989).

Methods: Cloning and sequencing of an Ac*-like element from* Pennisetum. Cloning and sequencing methods were as described in MacRae et al. (1990) and MacRae and Clegg (1992). The *Pennisetum Ac*-like element was aligned and compared with the maize *Ac* sequence using programs from the Wisconsin Genetics Computer Group (GCG) Package (Devereux et al., 1984). The average number of nucleotide substitutions per nucleotide site was calculated from the three parameter (3ST) model of Kimura (1981).

Southern blot analysis. The main conclusion from Southern blot analysis (data not shown) is that several diverse *P. glaucum* strains all hybridize with the ~2 kb pearl millet *Ac* subclone probe. Furthermore, all strains tested have the same, limited banding pattern when probed with the ~2 kb pearl millet *Ac* subclone. This suggests that the *Ac*-homologous DNA is in the same chromosomal location in all the diverse pearl millet strains and is not actively transposing. This would also imply that the pearl millet *Ac* element is "non-autonomous." However, this does not rule out the possibility of it being able to transpose in the presence of an active *Ac* element. The pearl millet *Ac* Southern hybridization pattern contrasts dramatically with Southern hybridization patterns from inbred maize lines containing active transposons. In such maize lines, one would see much greater variation in banding patterns between lines.

In addition to its Southern hybridization with *Pennisetum glaucum* strains, the pearl millet *Ac* ~2 kb probe also hybridizes to other *Pennisetum* and *Zea* species (data not shown). Hybridizing species include *Pennisetum setaceum* and *P. purpurensis*, as well as maize and teosinte lines.

Structural differences between maize Ac *and* Pennisetum Ac*-like elements.* There are two main structural differences between the sequenced region of the pearl millet *Ac*-like element and the corresponding region of the maize *Ac* element (Figure 8). The largest difference involves the second intron of the maize *Ac* element. This 71 bp segment of maize *Ac* is absent in pearl millet *Ac*, when the two elements are aligned by their DNA and deduced amino acid sequences (Figure 8, panel 2). The intron donor (GT) and acceptor (AG) sequences that are present in maize *Ac* represent the exact boundaries of the intron II "deletion" in pearl millet *Ac*. These well-defined boundaries suggest an exact removal (or addition) of the *Ac* second intron sequences during maize and pearl millet *Ac* evolution.

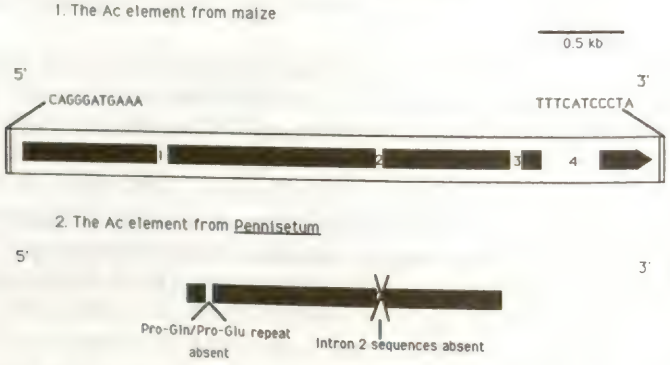


Figure 8. The structure of *Ac* in maize versus *Ac* in pearl millet (*Pennisetum glaucum*). **Top**: The 4.6 kb *Ac* element from maize is shown on top, by an open box. Black boxes inside the *Ac* element represent exons 1–5 of the *Ac* transcript. White spaces between the black boxes are introns and are numbered 1–4. The small open boxes at the ends of the element are terminal inverted repeats, whose sequences are shown. This upper panel shows the general structure of the maize *Ac* element, adapted from Fedoroff (1989a) Figure 1. It is not drawn to exact scale. **Bottom**: The sequenced portion of the pearl millet (*Pennisetum glaucum*) *Ac* element is shown in the bottom panel. It has been aligned with the corresponding 2041 bp of maize *Ac* sequences from the top panel. Alignment was confirmed at the amino acid level. Sequenced portions of exons 2 and 3 are shown in black. Two structural features differ between maize and pearl millet *Ac* elements: one is a Pro-Gln/Pro-Glu ten-fold amino acid repeat, present in maize but absent in pearl millet. This is represented by a 60-bp gap (white) in the 5′ end of pearl millet exon 2. The other structural difference is the absence in pearl millet *Ac* of any sequences corresponding to the second intron of the maize *Ac* element. This difference is represented by an **X** through the absent intron 2 region of the pearl millet *Ac*-like element. The published portion of the pearl millet *Ac* sequence (102 nucleotides) has been reported to EMBL, GenBank, and DDBJ Nucleotide Sequence Databases under the accession number X51894.

The second main structural difference between maize and pearl millet *Ac* elements involves a 60 bp segment of DNA encoding a Proline-Glutamine/Proline-Glutamic Acid amino acid repeat within the maize ORFa protein. The repeat, located at the 5′ end of exon two in maize *Ac*, is absent in pearl millet *Ac* (Figure 8, panel 2). In maize, this repeat is not required for transposase-to-DNA binding *in vitro*, however, its deletion abolishes the *Ac* transposase function *in vivo* (Kunze, pers. comm.). Therefore, the absence of such an amino acid repeat in pearl millet leads us to speculate that the pearl millet *Ac* element may also lack a transposase function. Why this particular amino acid repeat was apparently lost in pearl millet *Ac* leads to interesting speculations about which regions of a transposable element remain in the host genome and which critical portions will be deleted first in evolution.

The remaining structural differences between maize and pearl millet *Ac* elements consist of small insertions or deletions of a few base pairs, the majority of which do not disrupt the putative coding region. We do not know whether the pearl millet *Ac*-like element is a functional transposon: i.e., whether it has the ability to produce a transposase and/or serve as a substrate for transposase binding. Because the sequencing of the entire pearl millet *Ac*-like element is not yet completed, this necessitates the presentation here of an incomplete molecular characterization of the *Ac*-like element.

DNA sequence comparisons. In addition to structural comparisons, we can also examine the overall percent DNA similarity between maize and pearl millet sequences. From Table 1, 68 % of the DNA bases are identical between *Ac* from pearl millet and the corresponding region of *Ac* from maize. The *alcohol dehydrogenase 1* (*Adh1*) gene has also been sequenced in both maize (Dennis et al., 1984; Osterman and Dennis, 1989; Sachs et al., 1986) and pearl millet (Gaut and Clegg, 1991), and it serves as a relevant comparison to *Ac*. The *Adh1* gene from pearl millet has ten exons and nine introns, and, as expected, the percent DNA sequence identity differs for exon versus intron regions. In a comparison of all *Adh1* exons between maize and pearl millet, 87 % of the bases are identical (Table 1). In a comparison of all *Adh1* introns between maize and pearl millet, 57 % of the bases are identical (Table 1). Thus, our *Ac* element DNA sequence identity of 68 % (Table 1) falls in between the exon and intron sequence identities for the nuclear gene *Adh1*.

Table 1. The percent DNA sequence identity between maize and pearl millet sequences. The *Activator* (*Ac*) sequence, as well as the *alcohol dehydrogenase 1* (*Adh1*) exons and *Adh1* introns, are compared between maize and pearl millet. The percentage of the DNA bases that are identical between the two species is given.

Sequence	Identical DNA Bases
Ac	68%
Adh1 exons	87%
Adh1 introns	57%

We can also estimate the evolutionary distance between maize and pearl millet *Ac* element sequences using Kimura's (1981) three substitution model (Table 2). There is an average of 0.429 (± 0.020) nucleotide substitutions per site between maize and pearl millet *Ac* (Table 2). A total of 1887 base pairs were compared between maize and *Pennisetum* aligned sequences, in order to calculate Kimura's evolutionary distance. The *Adh1* gene can also be compared. The average number of synonymous substitutions per site for coding regions of *Adh1* between maize and pearl millet is 0.395 (± 0.051) and the average distance for *Adh1* intron sites is 0.256 (± 0.015) (Table 2). The *Ac* sequences thus appear to have accumulated nucleotide substitutions at about the synonymous rate and considerably faster than the intron rate. If we can assume that the *Adh1* divergence time is equivalent to the *Ac* divergence time, the above comparisons suggest that the *Ac* sequences have probably not been strongly constrained by natural selection. However, if there are several *Ac*-like elements in the pearl millet genome, we may be comparing a pearl millet element whose separation by duplication was even more ancient than the maize-pearl millet organismal separation presumably reflected in the *Adh1* comparison (see Figure 6 for an illustration).

Table 2. Kimura's (1981) evolutionary distance between maize and pearl millet DNA sequences. The evolutionary distance in number of nucleotide substitutions per site is estimated for three sequences, each compared between maize and pearl millet: the *Ac* sequence; the coding regions of the *Adh1* gene (synonymous sites only); and the intron regions of the *Adh1* gene. See text for interpretation.

Sequence	Nucleotide Sub./Site
Ac	0.429 (± 0.020)
Adh1 coding	0.395 (± 0.051)
Adh1 introns	0.256 (± 0.015)

Origins of Pennisetum Ac. One question we must still address is: "How did the pearl millet *Ac*-like element originate?" There are three potential answers: (1) by recent introgression between maize and pearl millet; (2) by close taxonomic relatedness of maize and pearl millet; and (3) as an ancient DNA sequence present in the ancestor of maize and pearl millet.

We can reject the first answer, that *Ac* entered the pearl millet genome via recent introgression from maize, because maize and pearl millet do not readily hybridize or produce fertile offspring. We can address the second and third possible answers by considering a phylogeny of the grasses, based not on *Ac* sequences, but on the *ribulose-1,5-bisphosphate carboxylase* (*rbc*L) gene from the chloroplast genome. The second answer, that the *Ac*-like element exists in pearl millet due to a close taxonomic relationship between maize and pearl millet, can also be rejected. Maize and pearl millet are two quite divergent grasses that have a deep root in the *rbc*L grass phylogeny (Doebley et al., 1990).

The third answer, that *Ac* is an ancient sequence present in the ancestor of maize and pearl millet, can be accepted, based on the following evidence. Using chloroplast DNA, *rbc*L sequence data and fossil evidence, plus assuming a molecular clock, we estimate that maize and pearl millet last shared a common ancestor between 25 and 42 million years ago (Doebley et al., 1990; Gaut and Clegg, 1991; MacRae et al., 1990). This estimated pearl millet/maize organismal divergence time and current reproductive isolation, coupled with the relatively high level of *Ac* sequence divergence, and the structural differences between maize and pearl millet *Ac* sequences, suggest the *Ac*-like element has not had a recent introduction into pearl millet. Instead, we must conclude *Ac* element sequences are very old and were present in the ancestral lineage from which maize and pearl millet descended (MacRae and Clegg, 1992).

Although *Ac*-like sequences are found in the genus *Zea*, in inbred maize (*Zea mays mays*) as well as in teosinte species (Johns, 1990), *Ac*-like sequences had not been previously found in a grass that is widely divergent from maize, nor had they been studied at the DNA sequence level outside of maize *Ac* until now. The conclusion that *Ac* element sequences are quite evolutionarily old and have existed for at least 25 million years, probably longer in grasses, is a novel one. A corollary prediction is that *Ac*-like sequences will be widely distributed in the grass family, Poaceae. Should this be found true, as seems probable (Vershinin et al., 1987), it would also support the hypothesis of an ancient origin for *Ac* sequences.

Active vs. non-active Ac *in pearl millet?* The final question to be addressed is: "Is the pearl millet *Ac*-like element active?" There are three lines of evidence suggesting it is not "active," i.e., is not capable of transposition: (1) Southern blot data show no RFLP variation among *Pennisetum* lines from diverse localities; (2) there is a high level of DNA sequence divergence between maize and pearl millet *Ac* sequences compared to maize and pearl millet *Adh1* sequences; and (3) the absence of intron II in pearl millet *Ac* compared to maize *Ac* suggests that the pearl millet element may be a pseudogene (see chapter 7 of Li and Graur, 1991).

There are, however, three lines of evidence indicating that the pearl millet *Ac*-like element may be "active," in the sense of being able to respond to a transposase: (1) Pearl millet *Ac*-derived amino acid sequences corresponding to maize *Ac* exons show strong conservation; and (2) the central maize *Ac* region is not required *in cis* for transposition (Coupland et al., 1988; Kunze and Starlinger, 1989). Recall that we have subcloned and sequenced the central region of pearl millet *Ac*, corresponding to that region of maize *Ac* beginning just after intron I and ending just before intron III. When the terminal regions of the pearl millet *Ac* element are also completely sequenced, we will look for evidence of transposase binding sites (Kunze and Starlinger, 1989), and other maize *Ac*-homologous sequences that would satisfy the requirements *in cis* for transposition-competence as a receptor element; (3) We are currently looking for any mRNAs that may be produced from the pearl millet *Ac*-like element, which would indicate its active transcription.

In conclusion, any use or study of plant transposable elements from a systematic perspective must concern itself with four main issues. First, the ages and evolutionary relationships of various transposon groups in plants must be assessed (MacRae and Clegg, 1992). Second, we must study the rates and modes of multiple-copy transposon sequence evolution, as well as the differing genomic distributions of transposons in related taxa. Third, it will be important to know the frequency at which horizontal transmission occurs in different transposon groups. Finally, the most important question remaining is the function of transposable elements in evolution, e.g., their possible roles as selfish DNA (Doolittle and Sapienza, 1980; Orgel and Crick, 1980), and/or as reservoirs of "genetic potential" to be activated in times of environmental stress (McClintock, 1984).

LITERATURE CITED

Arnheim, N. 1983. Concerted evolution of multigene families. Pp. 38–61 *in* M. Nei and R. K. Koehn (eds.), Evolution of Genes and Proteins. Sinauer Associates, Sunderland, Massachusetts.

Behrens, U., N. V. Fedoroff, A. Laird, M. Muller-Neumann, P. Starlinger, and J. Yoder. 1984. Cloning of the *Zea mays* controlling element *Ac* from the *wx-m7* allele. Molec. Gen. Genet. 194: 346–347.

Bishop, J. M. 1981. Enemies within: the genesis of retrovirus oncogenes. Cell 23: 5–6.

Brown, D. D., P. C. Wensink, and E. Jordan. 1972. A comparison of the ribosomal DNAs of *Xenopus laevis* and *Xenopus mulleri*: evolution of tandem genes. J. Molec. Biol. 63: 57–73.

Charlesworth, B. 1985. The population genetics of transposable elements. Pp. 213–232 *in* T. Ohta and K. Aoki (eds.), Population Genetics and Molecular Evolution. Japan Scientific Societies Press, Tokyo.

Chen, J., I. M. Greenblatt, and S. L. Dellaporta. 1987. Transposition of *Ac* from the *P* locus of maize into unreplicated chromosomal sites. Genetics 117: 109–116.

Chomet, P., D. Lisch, K. J. Hardeman, V. L. Chandler, and M. Freeling. 1991. Identification of a regulatory transposon that controls the *Mutator* transposable element system in maize. Genetics 129: 261–270.

_____, S. Wessler, and S. L. Dellaporta. 1987. Inactivation of the maize transposable element *Activator* (*Ac*) is associated with its DNA modification. E. M. B. O. J. 6: 295–302.

Coupland, G., B. Baker, J. Schell, and P. Starlinger. 1988. Characterization of the maize transposable element *Ac* by internal deletions. E. M. B. O. J. 7: 3653–3659.

Dennis, E. S., W. L. Gerlach, A. J. Pryor, J. L. Bennetzen, A. Inglis, D. Llewellyn, M. M. Sachs, R. J. Ferl, and W. J. Peacock. 1984. Molecular analysis of the *alcohol dehydrogenase* (*Adh1*) gene of maize. Nucl. Acids Res. 12: 3983–4000.

Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucl. Acids Res. 12: 387–395.

Doebley, J., M. Durbin, E. M. Golenberg, M. T. Clegg, and D.-P. Ma. 1990. Evolutionary analysis of the large subunit of carboxylase (*rbc*L) nucleotide sequence among the grasses (Gramineae). Evolution 44: 1097–1108.

Doolittle, R. F., D. F. Feng, M. S. Johnson, and M. A. McClure. 1989. Origins and evolutionary relationships of retroviruses. Quart. Rev. Biol. 64: 1–29.

Doolittle, W. F. and C. Sapienza. 1980. Selfish genes, the phenotype paradigm and genome evolution. Nature 284: 601–603.

Dover, G. A. 1982. Molecular drive: a cohesive mode of species evolution. Nature 299: 111–117.

Dowsett, A. P. 1983. Closely related species of *Drosophila* can contain different libraries of middle repetitive DNA sequences. Chromosoma 88: 104–108.

Fedoroff, N. V. 1989a. About maize transposable elements and development. Cell 56: 181–191.

_____. 1989b. Maize transposable elements. Pp. 375–411 in D. E. Berg and M. M. Howe (eds.), Mobile DNA. American Society for Microbiology, Washington, D.C.

_____, D. B. Furtek, and O. E. Nelson. 1984. Cloning of the bronze locus in maize by a simple and generalizable procedure using the transposable controlling element Activator (Ac). Proc. Natl. Acad. Sci. U.S.A. 81: 3825–3829.

_____, S. Wessler, and M. Shure. 1983. Isolation of the transposable maize controlling elements Ac and Ds. Cell 35: 235–242.

Flavell, A. J., E. Dunbar, R. Anderson, S. R. Pearce, R. Hartley, and A. Kumar. 1992. *Ty1-copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucl. Acids Res. 20: 3639–3644.

Freeling, M. 1984. Plant transposable elements and insertion sequences. Annual Rev. Pl. Physiol. 35: 277–298.

Gaut, B. S. and M. T. Clegg. 1991. Molecular evolution of *alcohol dehydrogenase 1* in members of the grass family. Proc. Natl. Acad. Sci. U.S.A. 88: 2060–2064.

Hobbs, H. H., M. S. Brown, J. L. Goldstein, and D. W. Russell. 1986. Deletion of exon encoding cysteine rich repeat of low density lipoprotein receptor alters its binding specificity in a subject with familial hypercholesterolemia. J. Biol. Chem. 261: 13114–13120.

Houck, M. A., J. B. Clark, K. R. Peterson, and M. G. Kidwell. 1991. Possible horizontal transfer of *Drosophila* genes by the mite *Proctolaelaps regalis*. Science 253: 1125.

Johns, M. A. 1990. Sequences related to the maize transposable element *Ac* in the Genus *Zea*. J. Molec. Evol. 30: 493–499.

Kidwell, M. G. 1992. Horizontal transfer of *P* elements and other short inverted repeat transposons. Genetica 86: 275–286.

Kimura, M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. Proc. Natl. Acad. Sci. U.S.A. 78: 454–458.

Kunze, R. and P. Starlinger. 1989. The putative transposase of transposable element *Ac* from *Zea mays* L. interacts with subterminal sequences of *Ac*. E. M. B. O. J. 8: 3177–3185.

_____, U. Stochaj, J. Laufs, and P. Starlinger. 1987. Transcription of transposable element *Activator* (*Ac*) of *Zea mays* L. E. M. B. O. J. 6: 1555–1563.

Li, W.-H. and D. Graur. 1991. Fundamentals of Molecular Evolution. Sinauer Associates, Sunderland, Massachusetts.

MacRae, A. F. and M. T. Clegg. 1992. Evolution of *Ac* and *Ds1* elements in select grasses (Poaceae). Genetica 86: 55–66.

_____, G. H. Learn, M. Karjala, and M. T. Clegg. 1990. Presence of an Activator (Ac)-like sequence in *Pennisetum glaucum* (pearl millet). Pl. Molec. Biol. 15: 177–179.

Masson, P., G. Rutherford, J. A. Banks, and N. V. Fedoroff. 1989. Essential large transcripts of the maize *Spm* transposable element are generated by alternative splicing. Cell 58: 755–765.

McClintock, B. 1945. Cytogenetic studies of maize and *Neurospora*. Carnegie Inst. Wash. Year Book 44: 108–112.

_____. 1946. Maize genetics. Carnegie Inst. Wash. Year Book 45: 176–186.

_____. 1948. Mutable loci in maize. Carnegie Inst. Wash. Year Book 47: 155–169.

_____. 1950a. The origin and behavior of mutable loci in maize. Proc. Natl. Acad. Sci. U.S.A. 36: 344–355.

_____. 1950b. Mutable loci in maize. Carnegie Inst. Wash. Year Book 49: 157–167.

_____. 1951a. Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. 16: 13–47.

_____. 1951b. Mutable loci in maize. Carnegie Inst. Wash. Year Book 50: 174–181.

_____. 1978. Mechanisms that rapidly reorganize the genome. Stadler Genet. Symp. 10: 25–48.

_____. 1984. The significance of responses of the genome to challenge. Science 226: 792–801.

Muller-Neumann, M., J. Yoder, and P. Starlinger. 1984. The DNA sequence of the transposable element *Ac* of *Zea mays*. Molec. Gen. Genet. 198: 19–24.

Nevers, P., N. Shepherd, and H. Saedler. 1986. Plant transposable elements. Advances Bot. Res. 12: 102–203.

Ohta, T. 1980. Evolution and Variation of Multigene Families. Springer-Verlag, Berlin.

Orgel, L. E. and F. H. C. Crick. 1980. Selfish DNA: the ultimate parasite. Nature 284: 604–607.

Osterman, J. C. and E. S. Dennis. 1989. Molecular analysis of the *Adh1-C*^{m} allele of maize. Pl. Molec. Biol. 13: 203–212.

Pohlman, R. F., N. V. Fedoroff, and J. Messing. 1984. The nucleotide sequence of the maize controlling element *Activator*. Cell 37: 635–643.

Qin, M. and A. H. Ellingboe. 1990. A transcript identified by *MuA* of maize is associated with *Mutator* activity. Molec. Gen. Genet. 224: 357–363.

Riley, M. and S. Krawiec. 1987. Genome organization. Pp. 967–981 *in* F. C. Neidhardt et al. (eds.), *Escherichia coli* and *Salmonella typhimurium*. Amer. Soc. for Microbiol., Washington, D.C.

Sachs, M. M., E. S. Dennis, W. L. Gerlach, and W. J. Peacock. 1986. Two alleles of maize *alcohol dehydrogenase 1* have 3′ structural and poly (A) addition polymorphisms. Genetics 113: 449–467.

Shapiro, J. A. 1992. Natural genetic engineering in evolution. Genetica 86: 99–111.

Sharp, P. M. and W.-H. Li. 1987. Molecular evolution of ubiquitin genes. Trends Ecol. Evol. 2: 328–332.

Sherratt, D. J., A. Arthur, R. Bishop, P. Dyson, P. Kitts, and L. Symington. 1983. Genetic transposition in bacteria. Pp. 59–74 *in* K. F. Chater, C. A. Cullis, D. A. Hopwood, A. A. W. B. Johnston, and H. W. Woolhouse (eds.), Genetic Rearrangement. Sinauer Associates, Sunderland, Massachusetts.

Varmus, H. E. 1983. Retroviruses. Pp. 411–503 *in* J. A. Shapiro (ed.), Mobile Genetic Elements. Academic Press, New York.

Vershinin, A. V., E. A. Salina, S. K. Svitashev, and V. K. Shumny. 1987. The occurrence of *Ds*-like sequences in cereal genomes. Theor. Appl. Genet. 73: 428–432.

Voytas, D. F. 1992. *Arabidopsis* and cotton (*Gossypium*) as models for studying *copia*-like retrotransposon evolution. Genetica 86: 13–20.

Wessler, S. R. 1988. Phenotypic diversity mediated by the maize transposable elements *Ac* and *Spm*. Science 242: 399–405.

Zimmer, E. A., S. L. Martin, S. M. Beverley, V. W. Kan, and A. C. Wilson. 1980. Rapid duplication and loss of genes coding for the a chains of hemoglobin. Proc. Natl. Acad. Sci. U.S.A. 77: 2158–2162.

GENETICS, DEVELOPMENT, AND THE MORPHOLOGICAL EVOLUTION OF MAIZE[1]

John Doebley[2]

ABSTRACT. Understanding the genetic basis of morphological evolution is an important concern of plant systematics. One issue surrounding morphological evolution is whether the evolution of a new trait typically involves a single gene of large effect or several loci with small and equal effects. Throughout this century, researchers have investigated this issue by studying the inheritance of morphological traits that distinguish related species or morphotypes of a single species. This work has usually involved the application of classical and quantitative genetics. Recently, it has become possible to gain a more detailed picture of the inheritance of morphological traits by combining quantitative genetic models with molecular marker analysis (isozymes or Restriction Fragment Length Polymorphisms, RFLPs). Using this approach, my colleagues and I have investigated the inheritance of several traits that characterize the dramatic morphological differences between maize (*Zea mays*) and its probable progenitor, teosinte (*Z. mays* subsp. *parviglumis*). Our results suggest that the evolution of the traits differentiating maize and teosinte have variable modes of inheritance, some controlled by a major locus (plus several minor loci) and others by several loci of approximately equal effect. This is the expected result if one accepts that evolution is opportunistic, utilizing whatever mutations (major or minor) arise within species. A second issue surrounding morphological evolution is understanding how genetic changes alter the course of morphogenesis, resulting in the differences in adult form that distinguish species. In this regard, the evolution of maize appears to have involved alterations in the time or place at which developmental programs are expressed, as opposed to the creation of novel developmental programs. For example, the evolution of soft glumes and paired-spikelets in maize appears to have involved alterations of morphogenesis that enabled the expression of male secondary-sex traits on a female background. Similarly, the origin of polystichous phyllotaxy in maize from distichous phyllotaxy in teosinte appears to have involved changes in the timing of the branching of the inflorescence primordium.

Understanding the genetic basis of the morphological differences among species is a critical concern of systematic biology. Such differences represent the traits that adapt organisms to specific niches, and they are the primary features upon which systems of classification are based. A fuller understanding of the genetic control of the morphological differences among plant species will add to our knowledge of how new traits evolve. Do new morphological features typically evolve in a stepwise fashion by sequential changes at numerous loci or can a change of large effect at a single locus produce a new trait that is subsequently refined by selection at more numerous modifier loci? An improved understanding of morphological evolution will also enable a fuller appreciation of systems of classification based on morphology. If, for example, a relatively small suite of mutable loci control floral development, one might expect parallelism for floral traits to be relatively frequent. Knowing the genetic control of different morphological features may also elucidate why some traits have greater utility for classification than others.

[1]I thank Drs. Elizabeth Kellogg, Jonathan Wendel and Korise Rasmusson for critical review of this paper. This work was supported by National Science Foundation grants (BSR88-06889 and BSR91-07175) and by Pioneer Hi-Bred, Intl. of Johnston, Iowa.

[2]Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, U.S.A.

In this chapter, I will review the efforts of my colleagues and me to unravel the genetic basis of the morphological evolution of maize from its probable progenitor, teosinte (Doebley et al., 1990; Doebley and Stec, 1991, 1993). I will consider the number of loci controlling some of the key traits that distinguish maize and teosinte and the relative magnitudes of their effects, i.e., whether they have large (major locus) or small effects (minor locus) on the trait. I will also consider how the actions of the loci we have identified alter the course of morphogenesis, thus producing the dramatic differences in adult morphology that distinguish maize and teosinte. The implications of our work for understanding the morphological evolution of both maize in particular and of plants in general will be discussed.

GENETICS OF MORPHOLOGICAL TRAITS

Analysis of the inheritance of morphological traits typically involves the study of closely related (cross-compatible) species or morphotypes of a single species. Segregating progenies of crosses of these different forms can reveal the number of genes controlling a morphological trait. For qualitative traits, one can categorize the progeny into discrete classes and demonstrate that the relative frequencies of these classes correspond to those expected for a given number of Mendelian loci. For example, a 3:1 ratio for alternate character-states of a trait in an F_2 population would indicate that the trait is controlled by a single locus with dominance. Although straightforward, there are limitations to this approach. *First*, traits that show continuous variation in segregating progenies are not amenable to this form of analysis. *Second*, there is a danger that one could unintentionally bias the categorization such that one obtains approximately the expected Mendelian ratio for a single locus even though the trait is controlled by several loci. *Third*, complex models postulating two or three loci with various modes of gene action can be found to fit virtually any ratio of progeny classes; however, such models are best considered hypothetical until confirmed by the analysis of advanced generations (F_3's, reciprocal backcrosses, isogenic lines, etc.) in which one separates the individual loci and analyzes their effects independently (Coyne and Lande, 1985; Ford and Gottlieb, 1990). Despite these concerns, this classical genetic approach to studying the genetics of discrete morphological traits works remarkably well, especially when analyzing mutants that arise within species. It is a valuable approach that should be applied more frequently by systematists to the morphological differences observed within and among species (Gottlieb, 1984; Ford and Gottlieb, 1992).

To study the inheritance of quantitative traits, one can use the Wright-Castle formula, which estimates the number of loci (n_E) controlling a quantitative trait as a function of the variance for the trait in the F_1 and F_2 populations as related to the difference in the parental means (Lande, 1981):

$$n_E = (\mu_{P_2} - \mu_{P_1})^2 / \{8(\sigma^2_{F2} - \sigma^2_{F1})\}$$

This approach has a number of assumptions that are not often met by real data (Zeng et al., 1990). One of these is that all alleles contributing positively to a trait come from one parent and that all alleles contributing negatively come from the other parent. This assumption is often violated (Edwards et al., 1987; also see below). Another important limitation of the Wright-Castle formula is that it estimates only the number of loci involved and not the relative magnitudes of their effects. Knowing the magnitude of the effect of each locus is critical information because evolutionists are interested in not only the number of genes that control a trait but also how much of the variance each explains. A trait controlled

by *n* polygenes (minor loci) will respond much differently to selection than one controlled by *n* - 1 polygenes plus a major locus. When one knows both the number of loci controlling a trait and the relative effects of each, then one begins to comprehend how the trait has evolved. Despite these limitations, the Wright-Castle formula provides a reasonable first estimate of the number of loci controlling differences in quantitative traits between inbred lines of domesticated species (Lande, 1981).

QTL-MAPPING

The approach that my colleagues and I have taken to elucidate the genetic control of the morphological evolution of maize is called QTL (quantitative trait locus) mapping (Edwards et al., 1987; Lander and Botstein, 1989). This method relies upon genetic linkage analysis and the availability of marker loci about every 20 centiMorgans throughout the genome. By QTL-mapping, one can estimate the number of loci controlling a trait, the chromosomal locations of these loci, the proportion of the phenotypic variance for which each locus accounts, and the mode of gene action of each locus (i.e., dominant, additive or overdominant).

QTL-mapping is applicable for comparisons of cross-compatible species that produce fertile first- and second-generation hybrids that lack severe cytological or other abnormalities (e.g., low fertility). One need not have inbred lines or special genetic stocks. This approach can be readily applied to natural species but is most easily done with self-compatible species for which one can generate a segregating F_2 population. With self-incompatible species, one can use backcrosses rather than F_2's; however, this places some limitations on data analysis. For example, a dominant locus would not be detected if the F_1 is backcrossed to the parent possessing the dominant allele.

Our QTL-mapping analyses in *Zea* demonstrate the steps typically involved in such a study. A single teosinte plant was used to pollinate a single plant of primitive maize. A single F_1 plant resulting from this cross was grown and self-pollinated to produce a segregating maize-teosinte F_2 population. Having used only a single F_1 plant means that the resulting F_2 progeny possess a maximum of two different alleles at any locus. If seeds of several F_1 plants were pooled, then there may be several alleles at each locus in the F_2 population. This would complicate the experiment, making some aspects of data interpretation uncertain. Plants of the segregating F_2 population were grown and assayed for their genotypes at molecular marker loci distributed over the entire nuclear genome at approximately 20 centiMorgan intervals (Fig. 1). The marker loci can be allozymes or cloned low-copy-number nuclear DNA sequences that reveal Restriction Fragment Length Polymorphisms (RFLPs). Codominant markers are preferable to dominant markers such as Randomly Amplified Polymorphic DNA (RAPDs) because the former enable one to distinguish both homozygous classes from the heterozygotes. Morphological traits of interest are also measured on each F_2 plant. Thus, one obtains a data matrix that includes the genotypes of the F_2 plants at numerous marker loci distributed throughout the genome and their phenotypes (measurements) for the morphological traits of interest.

Once the phenotypic and genotypic data are collected, there are two principal approaches to mapping QTLs. One method uses linear regression to examine the relationship between the phenotype for the quantitative trait and the genotypes at the individual marker loci (Edwards et al., 1987; Knapp and Bridges, 1990; Knapp et al., 1990). In the present case, the marker locus genotypes would be either homozygous teosinte (TT) or maize (MM), or heterozygous (MT). If there is a statistically significant association between the trait performance and the marker locus genotypes, one infers that a QTL is located near the marker locus. The R^2-values from the regression indicate the portion of the phenotypic variance for a

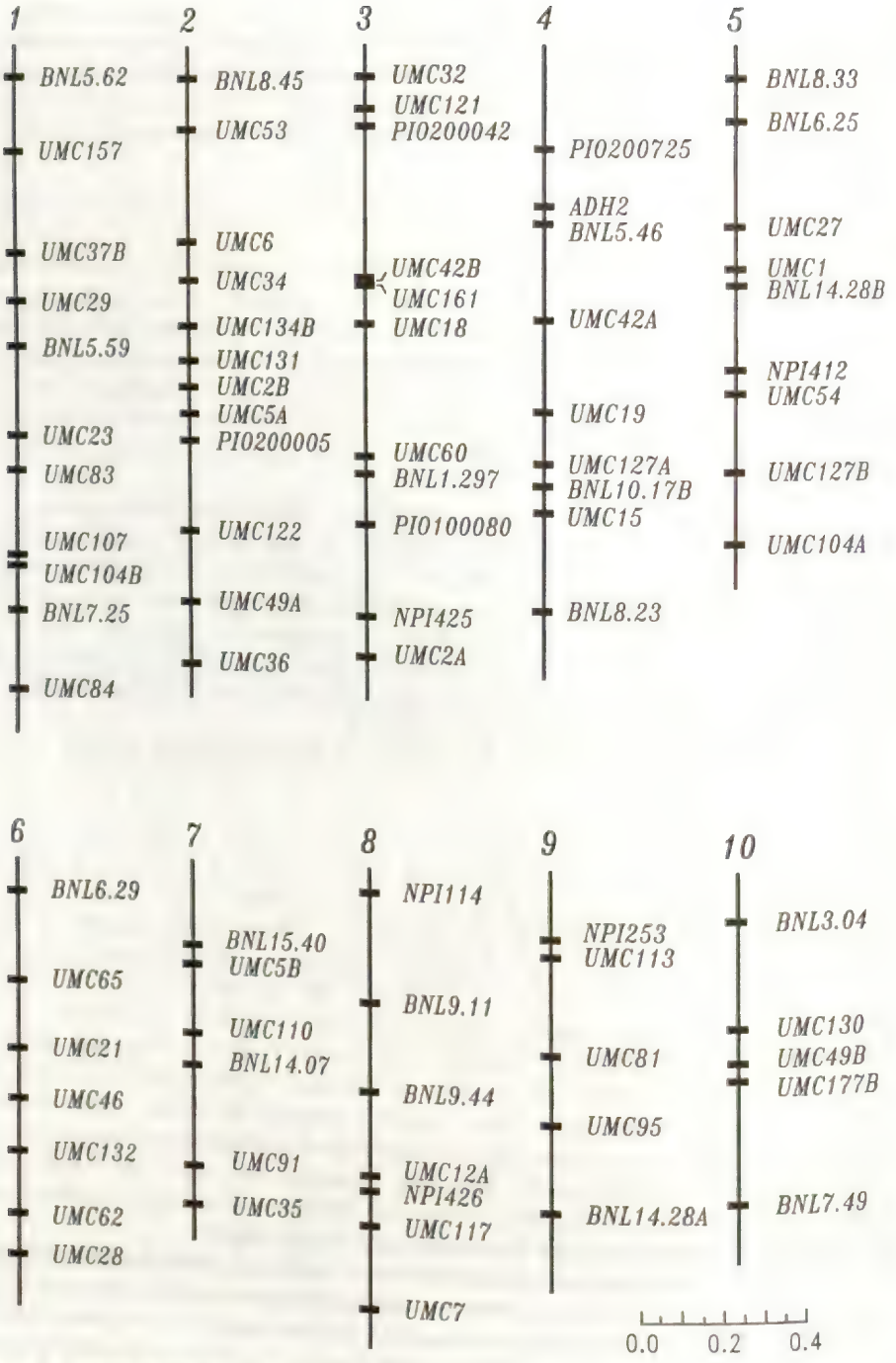


Figure 1. Diagram of the ten teosinte-maize chromosomes showing the distribution of molecular marker loci analyzed in the Race Reventador maize by Z. *mays* subsp. *parviglumis* teosinte F_2 population. Distances between the marker loci are shown as the recombination fraction (see scale). Prefixes indicate source of cloned probes as either Brookhaven National Laboratory (BNL), Native Plants Incorporated (NPI), Pioneer Hi-Bred International (PIO), or University of Missouri-Columbia (UMC).

trait that is explained by the QTL. A more recent method, called "interval mapping," involves calculating the ratio of the likelihood (odds) that there is a QTL to the likelihood that there is not a QTL at each position along the length of a chromosome (Lander and Botstein, 1989). These values are normally reported as the $\log_{10}$ of the "odds" ratio or LOD score. Where the LOD score exceeds a set threshold (significance level), one has evidence for the occurrence of a QTL.

QTL-MAPPING IN MAIZE-TEOSINTE POPULATIONS

My colleagues and I analyzed the inheritance of the key traits differentiating maize and teosinte in two F_2 populations (Doebley et al., 1990; Doebley and Stec, 1991, 1993). One of these populations was derived from a cross of Race Chapalote maize by *Z. mays* subsp. *mexicana* teosinte (C × M), and the other from a cross of Race Reventador maize by *Z. mays* subsp. *parviglumis* teosinte (R × P). Both crosses involved a primitive maize race and a form of teosinte that shows a close genetic relationship to maize. We employed primitive (small-eared) maize races because our goal was to analyze the genes involved in the early evolution of maize and not those involved in the evolution of modern (large-eared) from primitive maize (Doebley, 1992). Below, I review our results for five traits that differentiate maize and teosinte.

The first two traits concern plant architecture. Teosinte plants typically have elongated lateral branches that are terminated by male inflorescences, i.e., tassels (Fig. 2). This gives the teosinte plant the general appearance of a candelabra. In contrast, maize plants have short lateral branches that are terminated by female inflorescences, i.e., ears. Iltis (1983, 1987) was the first to discuss the significance of these differences in plant architecture in relationship to the origin of maize. My colleagues and I (Doebley et al., 1990; Doebley and Stec, 1991, 1993) studied both the length of the internodes on the lateral branches and the sex of the terminal inflorescence on the lateral branch (i.e., the percentage of the spikelets in the inflorescence that were staminate vs. pistillate). QTL-mapping revealed that internode length was controlled by multiple QTLs in both populations with the QTL of largest effect located on the long arm of chromosome 1 (i.e., 1L; Fig. 3). Both populations also possessed QTLs for internode length on chromosomes 3L, 5, and 6. Inflorescence sex was controlled by QTLs in several of the same locations as those for internode length, and again, the QTL of largest effect was on chromosome arm 1L (Fig. 3). In general, these two traits appear to be under multigenic control, although the QTL on chromosome arm 1L has reasonably large effect, accounting for 25–30% of the variance.

The third trait involves the degree of induration of the outer glume of the female inflorescence. In teosinte, these glumes are highly indurate and, along with the rachis internode, they form a hard casing that completely envelops the kernel (Fig. 4). This casing is called the "cupulate-fruitcase." Our QTL-mapping indicated that glume induration is controlled by several QTLs; however, in both F_2 populations, the QTL on chromosome arm 4S has a very large effect (controlling 42–50% of the variance), while the other QTLs have relatively small effects (Fig. 3). Since we first mapped the glume induration QTL on chromosome arm 4S, we have transferred this chromosome segment from teosinte into maize by backcross breeding (Doebley et al., 1992). Analysis of the resulting isogenic line showed that this chromosome segment contains a major Mendelian locus controlling glume induration. This locus, which we have termed *tga1* (teosinte glume architecture), affects several aspects of glume morphology including its degree of induration, its orientation relative to the axis of the ear, and its three-dimensional shape. Our results clearly indicate that the differences in glume architecture between maize and teosinte are controlled by a major locus and a series of minor QTLs.

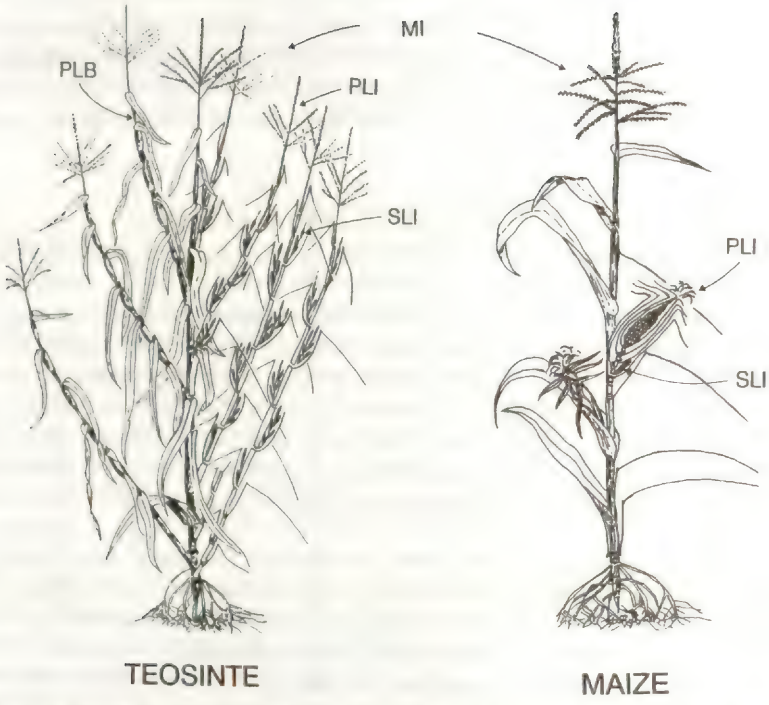


Figure 2. Mexican annual teosinte (left) with its candelabra-like growth form differs substantially from maize (right) in plant architecture. The long lateral branches of teosinte are each tipped by a tassel, while the short lateral branches of maize are tipped by ears. MI is the main inflorescence, PLI is the primary lateral inflorescence, SLI is the secondary lateral inflorescence, and PLB is the primary lateral branch. Adapted from Iltis (1987).

The fourth trait is the presence of single- vs. paired-spikelets in each cupule of the female inflorescence. Maize has two spikelets (one sessile and one pedicellate) in each cupule of its ears, while teosinte has only a single spikelet (the sessile one) in each cupule of its ears (Fig. 4). In our F_2 populations, this trait segregated in a quantitative fashion with the ears of most plants possessing paired-spikelets in some cupules and single-spikelets in others. QTL analysis of this trait gave similar results in both F_2 populations (Fig. 3). Single- vs. paired-spikelets was controlled by several QTLs; those with the largest effects reside on chromosome arms 1L and 3L. The inheritance of spikelet pairing in our F_2 populations seems best described as multigenic.

The final trait is inflorescence phyllotaxy. In teosinte, inflorescence phyllotaxy is strictly distichous or two-ranked (two rows of cupules), whereas inflorescence phyllotaxy in maize is polystichous or many-ranked (Fig. 4). The pattern of inheritance for inflorescence phyllotaxy was similar in the two populations with QTLs present on chromosomes 2, 3, 5 and 9. The C × M population showed a single major QTL on chromosome arm 2S plus several minor QTLs, while the R × P population showed two major QTLs (chromosome arms 2S and 5S) plus several minor ones. The reason that the C × M population revealed one major QTL and the R × P population two may be the fact that the maize parent of the C × M population had five-ranked phyllotaxy and the maize parent of the R × P population had six-ranked phyllotaxy. The six-ranked condition is a more advanced state, and thus one would expect that additional QTLs were involved in its evolution. The more primitive 5-ranked condition should show simpler inheritance. For this reason, the QTL on chromosome arm 2S, which is

common to both populations, may represent a locus of large effect that initially established four-ranked phyllotaxy in maize evolution. Subsequently, other QTLs led to the establishment of higher row numbers. We have further investigated the phyllotaxy QTL on chromosome arm 2S and found that it controls the shift from distichous to decussate (four-ranked) phyllotaxy (Doebley and Stec, unpublished).

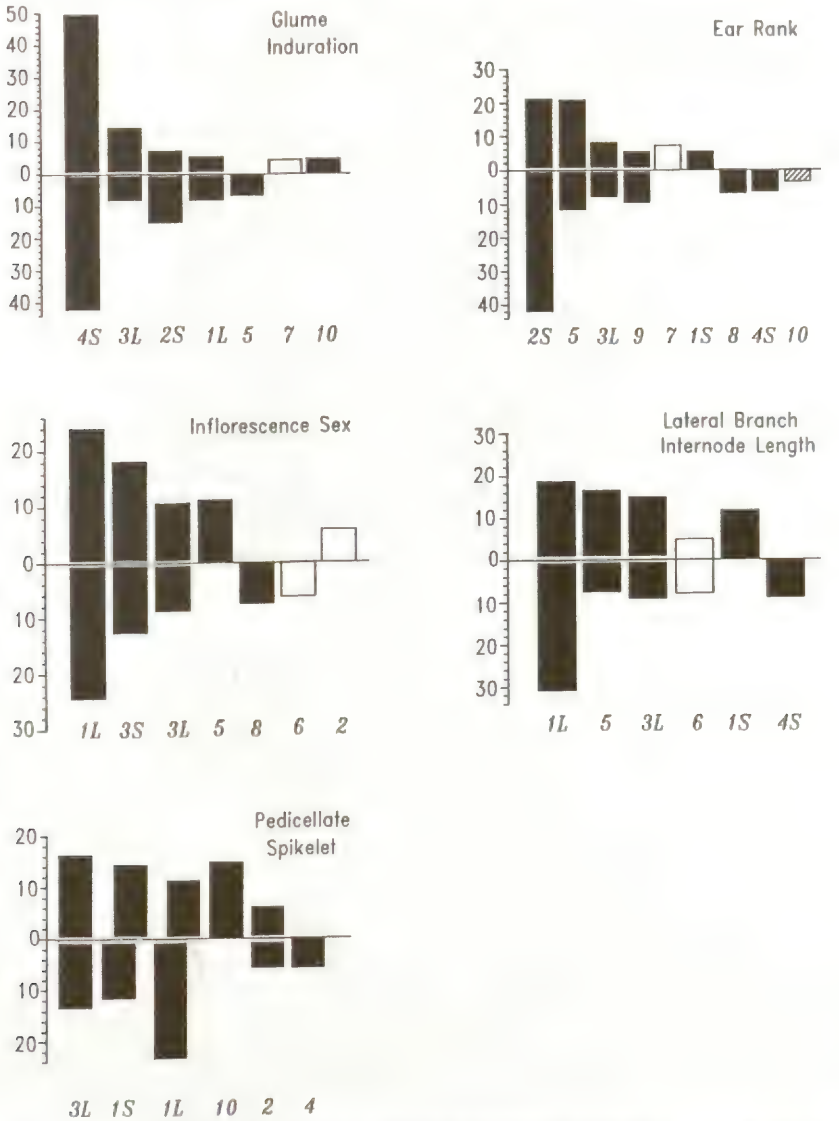


Figure 3. Column graphs showing the number and magnitude of significant associations (QTLs) between molecular marker loci and morphological traits. Results from the R × P population are shown above and the C × M population below the x-axis. The heights of the columns represent the R^2-values from the regression analysis expressed as a percentage. The numbers below each column are the chromosome or chromosome arm (*L* = long arm; *S* = short arm) on which the effect was detected. The columns from the two populations are aligned above/below one another if they putatively represent the same QTL. Columns are white if the effect of the QTL was in the wrong direction (i.e., a teosinte QTL that makes the plant more maize-like or vice versa), and they are shaded if there was apparent overdominance. From Doebley and Stec (1993).

Overall, QTL-mapping suggests that the pattern of inheritance varies considerably for different traits. Glume induration is controlled by a major locus plus modifiers. Similarly, the switch from two-ranked to four-ranked phyllotaxy involves a locus of large effect. Other traits, such as single- vs. paired-spikelets, are controlled by several QTLs of more equal effect. It is notable that no traits appear to be controlled by numerous (five or more) QTLs of equal and small effects.

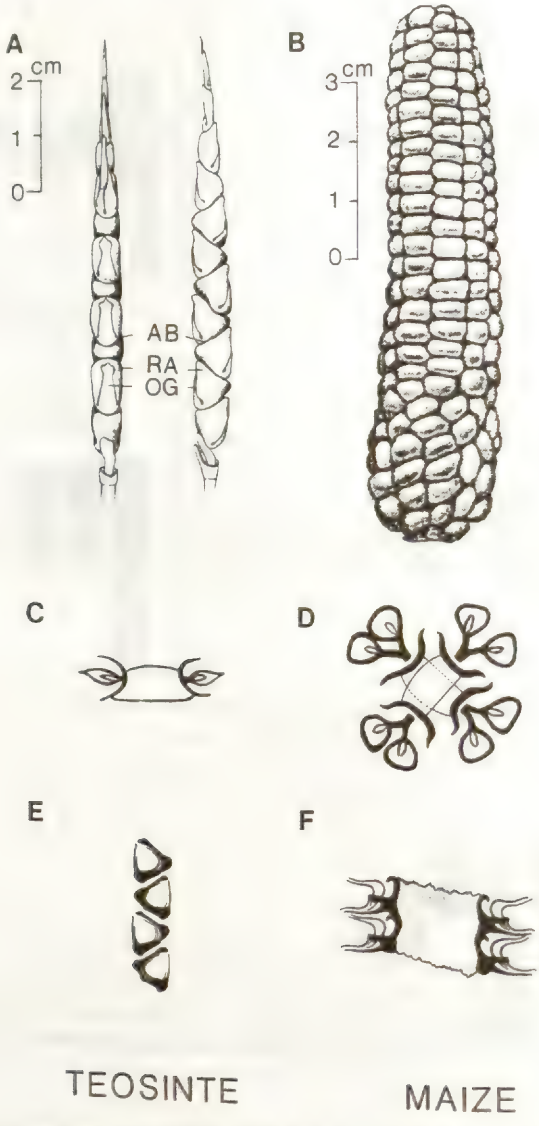


Figure 4. The most striking differences between maize and teosinte are in the architectures of their female inflorescences or ears. **A**. Teosinte ear: AB is the abscission layer that allows the teosinte ear to shatter when mature, OG is the outer glume, and RA is the rachis internode. **B**. Maize ear. **C–D**. Schematic transverse cross sections. **C**. Teosinte, showing two ranks of cupules with one kernel per cupule. **D**. Maize, showing four ranks of cupules with two kernels per cupule. **E–F**. Longitudinal cross sections. **E**. Four teosinte cupulate-fruitcases, showing the outer glume oriented upward, parallel to the axis of the ear. **F**. Maize, showing the outer glume oriented outward, perpendicular to the axis of the ear. Adapted from Iltis (1983) and Galinat (1969).

INTEGRATING GENETIC AND DEVELOPMENTAL MODELS

Identification of the genes or QTLs involved in an evolutionary transformation provides a foundation upon which one can construct models of how these genes alter the course of morphogenesis, thus producing the differences in adult form that one sees among species. Some understanding of how the QTLs we have identified alter inflorescence development can be proposed. I stress the preliminary nature of these models.

Soft Glumes: The male and female inflorescences of teosinte are distinguished by several **secondary-sex-traits** or morphological characters for which tassels and ears exhibit distinct character states. One of these is the degree of glume induration; tassels have soft glumes while those of ears are indurate. One feature of the evolution of maize is that its ear-glumes are softer (more like tassel-glumes) than the glumes of the teosinte ear. Thus, one can envision the evolution of the soft glumes of the maize ears as the expression of a male-secondary-sex trait on a female background (Galinat, 1983). One appeal of this model is that it does not require evolutionary novelty, i.e., the creation of a new developmental program to produce soft glumes in the ear. Rather, it requires only changes in the place of the expression of the tassel-glume developmental program. In this context, the locus controlling glume induration on chromosome arm 4S (*tga1*) can be viewed as having one of two functions. (1) *tga1* may represent a regulatory gene that activates a series of down-stream loci normally involved in the development of the ear-glumes (e.g., genes involved in glume induration). Under this scenario, if the function of *tga1* is disrupted or altered, then expression of the normal program for ear-glume development would be blocked and the default program could result in a softer, more male-like glume. (2) Alternatively, *tga1* may function to activate the program for tassel-glume development. Under this alternative, the maize allele at *tga1* would enable the expression of this program during both ear and tassel development.

This model for the evolution of soft glumes in the maize ear can be investigated in several ways. First, although the glumes of the maize ear superficially resemble those of the tassel, the resemblance may not survive a careful examination at the cellular level. One might find that the anatomical grounds of soft glumes in tassels and maize ears are unrelated. Second, one could examine the pattern of gene expression during the development of tassels and ears in maize and teosinte. If the model outlined above is correct, then some genes involved in the development of tassel-glumes should also be expressed during the development of maize ear-glumes but not during the development of teosinte ear-glumes.

Paired-Spikelets: Another secondary-sex-trait that distinguishes the ears and tassels of teosinte is the presence of single-spikelets at each node in the ear vs. paired-spikelets at each node in the tassel. In this regard, the maize ear exhibits another male secondary-sex-trait, paired-spikelets. The same model outlined above for the evolution of soft glumes could be applied to the origin of paired-spikelets in the maize ear, i.e., the origin of paired-spikelets in the maize ear results from the expression of a male-secondary-sex trait on a female background (Galinat, 1983). Again, one need not invoke an evolutionary novelty, but simply mutations that alter the place of expression of an existing developmental program. However, in contrast with the evolution of soft glumes, our current genetic data suggest that the evolution of paired-spikelets in the ear involved more complicated genetic changes.

Part of the reason that single- vs. paired-spikelets exhibits complex inheritance may be that this trait represents one facet of a more complex phenotype, i.e., inflorescence phyllotaxy. In teosinte, the formation of paired-spikelets during inflorescence development occurs as the result of branching or bifurcation of the inflorescence primordium (Sundberg and Orr, 1990). This same process is also involved in determining the phyllotaxy of the spikelets on the inflorescence (Cheng et al., 1983; Sundberg and Orr, 1990). Thus, it seems reasonable to propose that the inheritance of single- vs. paired-spikelets will depend on the

phyllotaxic background in which it is analyzed. In a distichous background, single- vs. paired-spikelets may exhibit relatively simple inheritance (one or two genes of large effect). However, in a background in which loci controlling phyllotaxy are also segregating (e.g., a maize-teosinte F_2 population), the inheritance of single- vs. paired-spikelets may appear more complex. In a sense, the adult traits, phyllotaxy and single- vs. paired-spikelets, may be different adult manifestations of a common developmental program or separate but interacting programs. This view is supported by the fact that both adult traits are influenced by QTLs in the same region of the genome (Fig. 3).

Inflorescence Phyllotaxy: The changes in morphogenesis involved in the origin of polystichy are not well understood. Several authors have suggested that "twisting" of the inflorescence axis is involved and have made this twisting a part of their overall models for the origin of the maize ear (Collins, 1919; Galinat, 1978; Iltis, 1983). This view, which is based only on observations of adult morphology, has been challenged by developmental studies demonstrating that there is no twisting during inflorescence development (Cheng et al., 1983; Sundberg and Orr, 1990). These developmental studies reveal that changes in the pattern of branching or bifurcation of the inflorescence primordium are involved. Our genetic analyses support the latter view.

During inflorescence development in teosinte, the terminal meristem of the inflorescence primordium produces branch primordia one at a time with each oriented 180° around the axis of the inflorescence relative to the branch primordium above and below (Sundberg and Orr, 1990). These primary branch primordia subsequently bifurcate to produce the sessile and pedicellate spikelet primordia (secondary branches). This pattern of branching results in sessile-pedicellate spikelet pairs arranged in a distichous pattern. In the teosinte tassel, both the sessile and pedicellate spikelets develop fully; however, in the teosinte ear the pedicellate spikelet is aborted soon after its initiation (Sundberg and Orr, 1990).

Our genetic analyses enable us to identify tentatively two discrete alterations of the normal teosinte inflorescence development that are involved in the origin of polystichy. First, among our F_2 plants, we identified some plants in which one of the spikelet primordia underwent an additional round of bifurcation, producing three spikelets at a single node (Fig. 5A). The occurrence of this additional round of bifurcation need not involve a novel developmental program, but may simply entail the expression of the existing program for bifurcation for an extended period of time. QTLs on chromosomes 1 and 5S largely control this process. Second, we identified some F_2 plants in which the terminal meristem of the inflorescence primordium produces two spikelet-pair primordia simultaneously (at a single node) on opposite sides of the primordium, these oriented 90° relative to those at the nodes above and below (Fig. 5B). This pattern of branching results in decussate phyllotaxy. This switch between distichous and decussate phyllotaxy is largely controlled by the major QTL for ear rank on chromosome arm 2S (Fig. 3). Preliminary data suggest that this QTL may segregate like a major (qualitative) locus when studied in an isogenic background.

Plant Architecture: While teosinte typically exhibits a candelabra-like growth form, under dry conditions or in shallow soils stunted plants in which the lateral branches fail to elongate can occur (see Iltis, 1987, fig. 19.1). The shorter lateral branches of these plants may be terminated by either female or mixed-sex inflorescences. The ability of teosinte to switch between these growth forms gives it the amplitude necessary to adapt to the different microhabitats in which the seed might germinate. In moist sites, tall plants with long lateral branches tipped by tassels produce both abundant seed and pollen. In dry or shallow-soiled sites or in dense stands, unbranched plants with few ears guarantee production of at least a few seeds.

From a developmental genetic perspective, one might argue that the switch between these two growth forms would involve perhaps dozens of genes. However, if each of these genes needs to respond independently to the environmental stimulus, then a coordinated response could be difficult to orchestrate. On the other hand, if a single or small number of regulator loci respond to the environmental stimulus, and subsequently activated other loci necessary to produce the appropriate phenotype, a coordinated response would be likely.

Unlike teosinte, maize has less phenotypic plasticity and does not switch between these two growth forms; its lateral branches are always short and terminated by ears. In a sense, maize seems genetically fixed for the depauperate teosinte growth form described above. The origin of this condition in maize may have involved mutations in the hypothetical regulatory locus described above. Our QTL mapping identified chromosome arm 1L as the region of the genome that controls much of the variance in plant architecture. In this same region of the genome, there is a locus in maize called *teosinte branched* (*TB1*) that controls a switch between short branches tipped by ears (*Tb1*, wild type) and long lateral branches tipped by tassels (*tb1*, mutant). My colleagues and I have previously hypothesized that *TB1* may be a key locus in the evolution of maize plant architecture (Doebley and Stec, 1991). Perhaps, *TB1* functions in teosinte as a regulatory locus that responds to the environment and then activates the proper down-stream loci to produce the appropriate plant architectural phenotype. During the evolution of maize, a mutation at *TB1* (and other loci) could have altered this locus such that maize can only produce short lateral branches tipped by ears.

IMPLICATIONS FOR MORPHOLOGICAL EVOLUTION IN PLANTS

Several themes have emerged from the analysis of the genetics of the morphological evolution of maize that may have broader implications for plant evolution. However, caution must be exercised in applying our results more broadly since evolution under domestication may differ appreciably from that under natural selection.

Some authors have proposed that plant evolution frequently involves single major loci that establish a new trait in a single step (Hilu, 1983; Gottlieb, 1984), while others have contended that the role of single major loci is rare and that the establishment of a new trait normally involves several loci with individually small effects (Charlesworth et al., 1982; Coyne and Lande, 1985). Results with maize suggest that the evolution of a new trait may involve genetic changes along the entire spectrum from the single major locus (plus modifiers) to the several loci of relatively small effect. In my view, this result is not surprising since evolution is opportunistic, working with whatever types of mutations arise within species. The types of mutations that can be involved in the evolution of a new trait are restricted, in that their effects must be large enough to provide a selective advantage and they must lack significant deleterious pleiotropic effects.

While our evidence from maize is compatible with a mode of evolution for several traits involving one or two major loci plus modifiers, this interpretation does not necessitate that genes with major effects resulted from single major mutations. A series of stepwise mutations at a single locus could create alleles with dramatically different effects, although as the result of incremental changes rather than a single step (mutation). Thus, the data for maize do not resolve whether major loci (QTLs) involved in maize evolution arose by (1) single mutations that dramatically altered the function of the gene, or (2) an incremental process composed of a series of small steps (mutations) at a single locus. I predict that once the genes involved in morphological evolution are analyzed at the molecular level it will be found that multiple mutations within a single locus are a common feature of evolution. Such changes might include alteration in both 5′ regulatory sequences and the coding region.

While the model that loci with large effects played a central role in the evolution of maize is still hypothetical, it is interesting to consider whether this mode of evolution is restricted to crop species or might be a more general feature of plant evolution (Coyne and Lande, 1985). The early evolution of a crop may be envisioned as a shift from a highly competitive (wild) niche to a new, essentially unoccupied niche (the cultivated field) with much reduced competition. Wright (1982) proposed that species that invade unoccupied niches experience reduced competition, and therefore are able to use mutations with drastic effects even if these mutations are accompanied by some unfavorable pleiotropic effects. If this view is correct, then the evolution of maize, rather than being a special case, may represent a common mode of evolution—the invasion of a new niche. Similarly, the establishment of a new trait under reduced competition might commonly involve few loci with large effects.

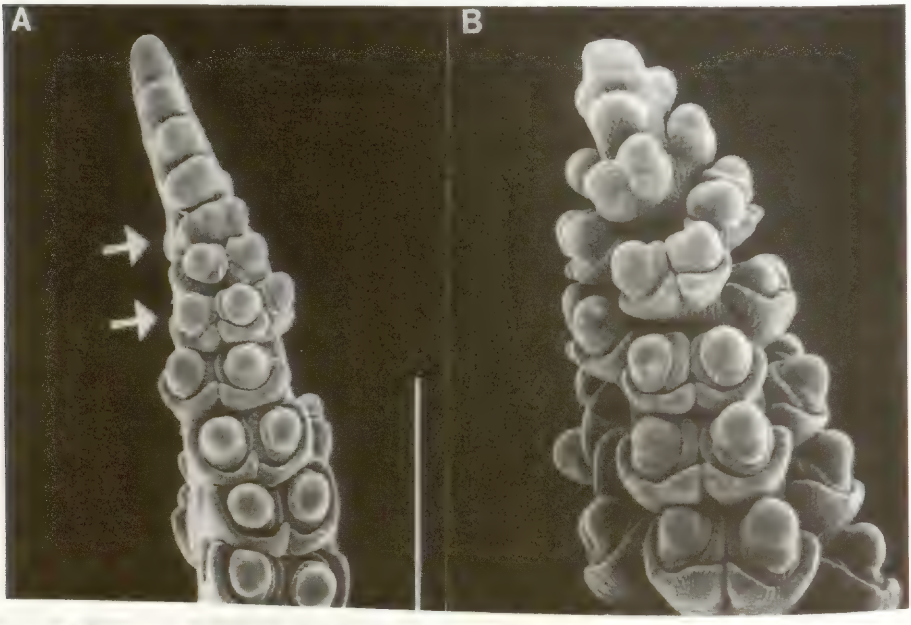


Figure 5. Inflorescence primordia from F_3 plants derived from a cross of Race Reventador maize by *Z. mays* subsp. *parviglumis* teosinte. **A**. Inflorescence primordium showing spikelet primordia which have gone through an additional round of branching (bifurcation), resulting in three spikelets at a single node (arrows). This is a novel phenotype found in neither parental taxa. **B**. Inflorescence primordium showing a decussate pattern of initiation of the spikelet-pair primordia. Bar = 500 microns.

Finally, our results provide some evidence that novel phenotypes may arise as the result of recombination of different developmental programs in hybrid populations. As discussed above, maize has two spikelets in each cupule of its ear and teosinte has one spikelet. Among the maize-teosinte F_2 populations, we recovered some plants with three spikelets in a single cupule (Fig. 5A). This is a novel phenotype found in neither of the parents. The generation of such novel phenotypes in hybrids may be an important component of recombinational or hybrid speciation and may help explain why hybrid taxa are not always intermediate between their parents (Rieseberg, this volume; McDade, this volume).

FUTURE PROSPECTS

The research summarized in this chapter represents only the beginning of our attempt to elucidate the morphological evolution of maize from teosinte. Having identified some of the genes and QTLs involved in this transformation, we may now begin to investigate more precisely the role of these loci in controlling morphogenesis. This will be accomplished in part by creating a series of isogenic lines in which the QTLs that differentiate maize and teosinte are isolated so that they can be analyzed in a uniform genetic background. Ultimately, our goal is to molecularly clone these loci and characterize their effects at the molecular, cellular and tissue levels. If this can be accomplished, then it may at long last be possible to understand how the dramatic transformation of teosinte into maize took place and to distill from maize evolution principles that may apply more broadly to morphological evolution.

LITERATURE CITED

Charlesworth, B., R. Lande, and M. Slatkin. 1982. A neo-Darwinian commentary on macroevolution. Evolution 36: 474–498.

Cheng, P. C., R. I. Greyson, and D. B. Walden. 1983. Organ initiation and the development of unisexual flowers in the tassel and ear of *Zea mays*. Amer. J. Bot. 70: 450–462.

Collins, G. N. 1919. Structure of the maize ear as indicated in *Zea-Euchlaena* hybrids. J. Agric. Res. 17: 127–135.

Coyne, J. and R. Lande. 1985. The genetic basis of species differences in plants. Amer. Naturalist 126: 141–145.

Doebley, J. 1992. Mapping the genes that made maize. Trends in Genetics 8: 302–306.

_____, J. Dorweiler, and J. Kermicle. 1992. Teosinte glume architecture (*tga1*), a locus from teosinte. Maize Gen. Newsletter 66: 95–96.

_____and A. Stec. 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics 129: 285–295.

_____and _____ . 1993. Inheritance of the morphological differences between maize and teosinte: comparison of results for two F_2 populations. Genetics 134: 559–570.

_____, _____, J. Wendel, and M. Edwards. 1990. Genetic and morphological analysis of a maize-teosinte F_2 population: implications for the origin of maize. Proc. Natl. Acad. Sci. (U.S.A.) 87: 9888–9892.

Edwards, M. D., C. W. Stuber, and J. F. Wendel. 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics 116: 113–125.

Ford, V. S. and L. D. Gottlieb. 1990. Genetic studies of floral evolution in *Layia*. Heredity 64: 29–44.

_____and _____ . 1992. *Bicalyx* is a natural homeotic floral variant. Nature 358: 671–673.

Galinat, W. C. 1969. The evolution under domestication of the maize ear: string cob maize. Mass. Agric. Exper. Sta. Bull. 577: 1–19.

_____. 1978. The inheritance of some traits essential to maize and teosinte. Pp. 93–111 *in* D. B. Walden (ed.), Maize Breeding and Genetics. John Wiley and Sons, NY.

_____. 1983. The origin of maize as shown by key morphological traits of its ancestor, teosinte. Maydica 28: 121–138.

Gottlieb, L. D. 1984. Genetics and morphological evolution in plants. Amer. Naturalist 123: 681–709.

Hilu, K. W. 1983. The role of single-gene mutations in the evolution of flowering plants. Evol. Biol. 26: 97–128.

Iltis, H. H. 1983. From teosinte to maize: the catastrophic sexual transmutation. Science 222: 886-894.

_____. 1987. Maize evolution and agricultural origins. Pp. 195–213 *in* T. Soderstrom, K. Hilu, C. Campbell, and M. Barkworth (eds.), Grass Systematics and Evolution. Smithsonian Inst. Press, Washington, D. C.

Knapp, S. J. and W. C. Bridges. 1990. Using molecular markers to estimate quantitative trait locus parameters: power and genetic variances for unreplicated progeny. Genetics 126: 769–777.

_____, _____, and D. Birkes. 1990. Mapping quantitative trait loci using molecular marker linkage maps. Theor. Appl. Genet. 79: 583–592.

Lande, R. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99: 541–553.

Lander, E. S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199.

Sundberg, M. D. and A. R. Orr. 1990. Inflorescence development in two annual teosintes: *Zea mays* subsp. *mexicana* and *Z. mays* subsp. *parviglumis*. Amer. J. Bot. 77: 141–152.

Wright, S. 1982. Character change, speciation, and higher taxa. Evolution 36: 427–443.

Zeng, Z.-B., D. Houle, and C. C. Cockerham. 1990. How informative is Wright's estimator of the number of genes affecting a quantitative character. Genetics 126: 235–247.

THE FOSSIL HISTORY OF BRANCHING: IMPLICATIONS FOR THE PHYLOGENY OF LAND PLANTS[1]

Gar W. Rothwell[2]

ABSTRACT. Recent paleobotanical discoveries relating to the conducting tissues and to the gametophytes of the most ancient land plants have prompted reevaluation of the criteria by which major clades of embryophytes are defined. Branching of the sporophyte is one of the most important of these criteria, and it conforms to basic patterns that are the result of a small number of variables. These include (1) a sporophyte that is either unbranched or branched, (2) branching that is either apical or lateral (axillary), and (3) branching that produces either unipolar or bipolar growth. Each of these variables has a profound impact on the growth potential of the plant. When combined with characters of the life cycle, the developmental patterns thus produced can be recognized as "models of growth." In this paper, several such "models" are introduced and characterized (i.e., Bryophyte Model, Cooksonioid Model, Psilotioid Model, Selaginelloid Model, Isoetoid Model, and Cotyledonoid Model). Stratigraphic distributions of these models are reviewed and related to the origins of major groups of land plants.

INTRODUCTION

Important roles of paleontology in systematic biology are to characterize new specimens of extinct organisms, to develop fresh insights into questions of relationships, and to provide the impetus for redefining concepts in ways that help increase our understanding of phylogenetic patterns. Sometimes paleontological discoveries are spectacular and unexpected, revealing the existence of new taxa, of highly specialized organs, of sophisticated developmental or reproductive mechanisms, or of intimate biological interactions. Other times, such characterizations involve the reevaluation of data and concepts that previously have been considered well understood.

Patterns of branching have been thoroughly characterized for most vascular plants, and several branching patterns are recognized as being correlated with major systematic groups (Gifford and Foster, 1989; Stewart and Rothwell, 1993). For example, apical branching may be equal (i.e., isostomous), unequal (i.e., anisostomous), or pseudomonopodial, and is characteristic of free-sporing pteridophytes. In contrast, axillary branching typifies seed plants and may be monopodial or sympodial. Variations in the pattern of branching also have been identified, and combinations of these variations have been used to define characteristic architectures of growth (Hallé, Oldeman, and Tomlinson, 1978; Trivett, 1991). Were these aspects of branching the major focus of this work, few additional comments would be in order, and the reader would be well advised to obtain this information from any of the numerous excellent summaries that currently are available (e.g., Tomlinson, 1983).

[1]This work has benefited significantly from helpful discussions with Dr. Eric Karrfalt, and with Dr. Dale Vitt, University of Alberta. I thank Dr. Adrian D. Bell, University of Wales, Bangor, for prompting the initiation of this work, Dr. Diane Edwards, University of Wales College of Cardiff, for supplying the photograph in Figure 1, Dr. David Barrington, University of Vermont, for supplying the photograph in Figure 3, Dr. Winfried Remy, Geologisch-Palaontologischen Institut, Münster, for leading the field trip upon which *Sciadophyton* was collected, and Dr. Ruth Stockey, University of Alberta for making available space and facilities at the University of Alberta, where this paper was written. Dr. Peter R. Crane, Field Museum of Natural History, Chicago, and an anonymous reviewer made valuable suggestions for improvement during revision of the manuscript. Received for publication October 29, 1992.

[2]Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701, U.S.A.

There are, however, additional and perhaps more fundamental facets of branching that traditionally have received less attention than those discussed above. Some of these define and explain the architectural constraints within which plants develop, and several have potentially profound systematic implications. When considered in conjunction with recent paleobotanical discoveries, patterns of branching resulting from these facets provide an opportunity to reassess our understanding of the diversity of growth, to consider the systematic implications of growth form, and to reevaluate the characters by which we define major clades of land plants.

In this paper, three important parameters of branching are emphasized and defined as systematic characters. These characters are polarized and combined to define alternative modes of branching among land plants. When considered in conjunction with major types of embryophyte life cycles, modes of branching provide the basis for recognizing fundamental models of growth for land plants (Figure 1). Several models of growth are defined, named, and the developmental changes from one model to another are evaluated (Table 1) to emphasize synapomorphies (Figure 12) that may be of value in evaluating hypotheses of embryophyte phylogeny.

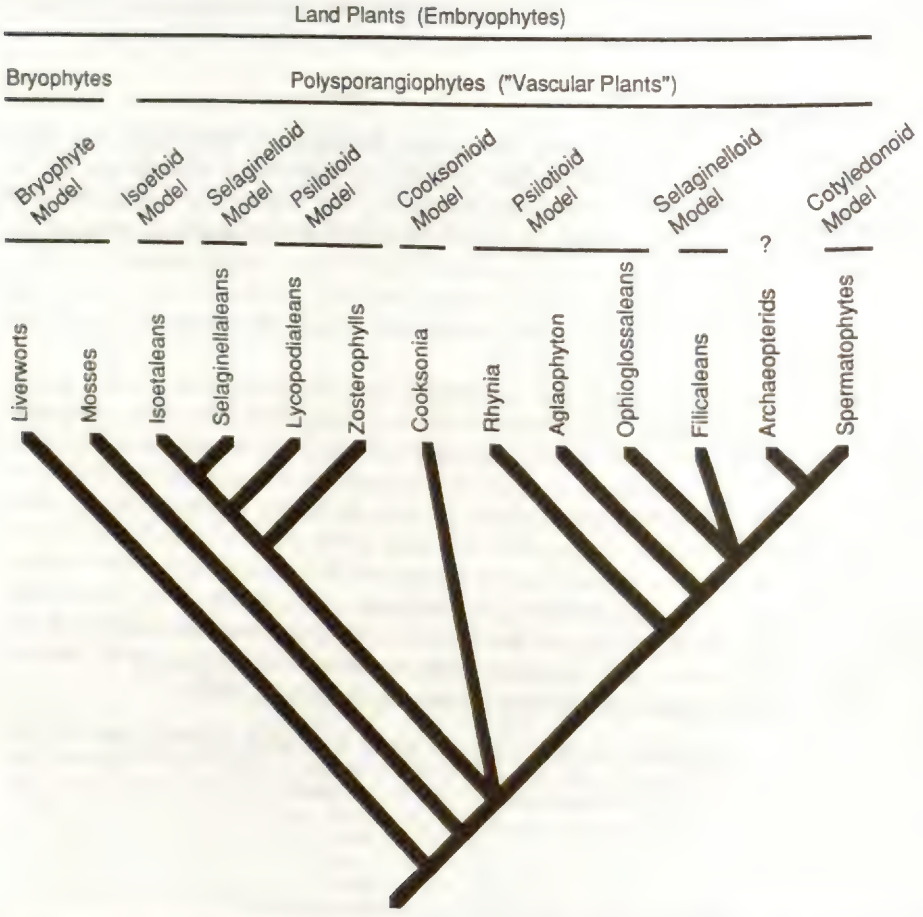


Figure 1. Systematic relationships of land plants (tree modified from Crane, 1991), showing distribution of models of growth as defined in this paper. See text for details.

Table 1. Basic Models of growth in Land Plants.

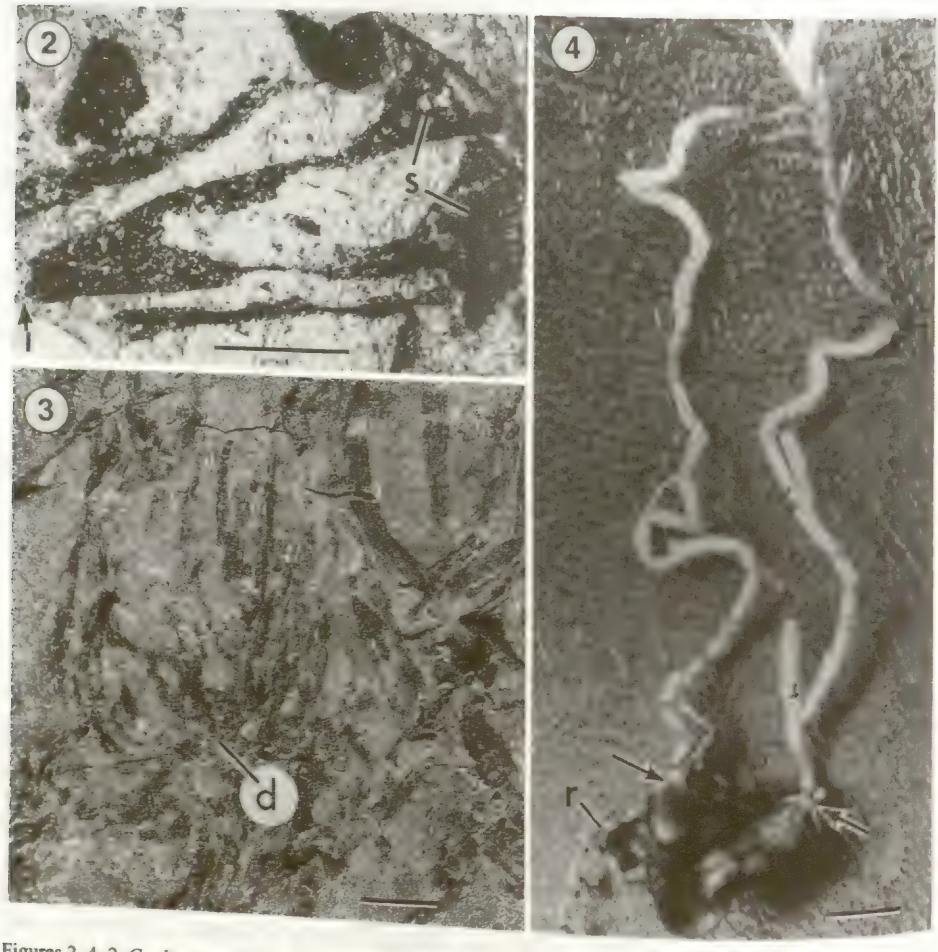
Model	First Occurrence	Change From
1. Bryophyte Model	Lower Devonian?	Cooksonioid Model, Loss of Branching
2. Cooksonioid Model	Lowermost Lower Devonian	Bryophyte Model, Potential for Branching
3. Psilotioid Model	Lower Devonian	Cooksonioid Model, Indeterminate Growth
4. Selaginelloid Model	Upper Devonian	Psilotioid Model, Independent Sporophyte
5a. Cotyledonoid Model	Lower Carboniferous?	Selaginelloid Model, Bipolar Growth-Cotyledonary Embryo Axillary Branching
5b. Isoetoid Model	Upper Devonian?	Selaginelloid Model, Bipolar Growth-Rhizomorph

WHAT IS A VASCULAR PLANT?

Among land plants with multicellular sporangia and gametangia (i.e., embryophytes), vascular plants traditionally have been distinguished from bryophytes (Figure 1) by two criteria: (1) the nature of the conducting tissue, and (2) the nature of the life cycle (Raven, Evert, and Eichhorn, 1992; Stewart and Rothwell, 1993). Sporophytes of vascular plants typically have water-conducting cells with differentially thickened secondary walls (i.e., tracheary elements), whereas in bryophytes walls of the conducting cells (when present) are uniformly thin (Taylor and Taylor, 1993). In bryophytes the sporophyte phase of the life cycle is borne on the gametophyte phase and is dependent upon the gametophyte phase for both support and nutrition (Stewart and Rothwell, 1993). In contrast, the sporophyte phase of vascular plants is considered to be independent of the gametophyte beyond the embryonic stage. However, recent studies of the most ancient land plants from Silurian and Devonian deposits have blurred these distinctions and have prompted critical reevaluation of the criteria by which we classify embryophytes (Kenrick and Crane, 1991; Remy, Gensel, and Hass, 1993).

One recent focus of paleobotanical study has developed from the discovery of gametophytes in Lower Devonian sediments (Remy and Remy, 1980; Remy et al., 1980a). Some gametophytes are compressed and reveal overall morphology of the plant (Figure 3; Remy et al., 1980b; Remy, Schultka, and Hass, 1991; Remy, Hass, and Schultka, 1991), whereas others are anatomically preserved and display remarkably complete preservation of gametangia (Remy and Hass, 1991). Both have features of the conducting strands, which reveal that vascular tissues were not restricted to the sporophyte phase of the life cycle in primitive land plants. These discoveries emphasize that gametophytes for some of the most ancient species of land plants were large, much-branched axial prothalli (Figure 3) with relatively complex tissues and organography (Remy, 1982; Kenrick, Remy, and Crane, 1991; Remy, Gensel, and Hass, 1993).

Another series of recent paleobotanical investigations profoundly impacts the second criterion by which we traditionally define vascular plants. Beginning with the discoveries that putative tracheids of some Devonian land plants have either a unique fine structure (Hueber, 1982) or uniformly thin walls (Edwards, 1986), accumulating paleobotanical evidence is demonstrating a heterogeneity of ultrastructure among cells that traditionally have been used to define vascular tissue (Edwards and Edwards, 1986; Kenrick, Edwards, and Dales, 1991). Up to the present, four distinctly different patterns of construction have been recognized for the water-conducting cells among plants that previously were considered to be the most ancient representatives of the Tracheophyta (Kenrick and Crane, 1991; Remy, Gensel, and Hass, 1993). While the phylogenetic implications of these discoveries are not yet fully understood (Hemsley, 1992), it is clear that the tracheid, as traditionally defined, can no longer be considered as a synapomorphy that defines a clade commonly known as vascular plants.



Figures 2–4. 2. *Cooksonia* sp., branched sporophyte showing two terminal sporangia (at s) and broken proximal end of axis (at arrow); scale = 1 mm. 3. *Sciadophyton* sp., with branching gametangiophores extending from central disk (at d); scale = 5 mm. 4. *Lycopodium* individual that consists of a long-lived subterranean gametophyte (proximally) from which two sporophytes extend distally. Arrows indicate positions at which sporophytes attach to gametophyte. Note adventitious root (at r); scale = 5 mm.

VARIATIONS IN PATTERNS OF BRANCHING AMONG LAND PLANTS

There are three parameters of branching that contribute to the fundamental growth form of land plants and that produce morphologies that are strongly correlated with major systematic groups. These are:

1. Sporophyte Branched or Unbranched. In their recent analysis of embryophyte systematics, Kenrick and Crane (1991) emphasize that the sporophytes of bryophytes produce only one sporangium. This is because such sporophytes do not branch. The sporangium is terminal in mosses and liverworts (when present). Therefore, the sporophyte has determinate growth that ceases upon production of the sporangium. The basal meristem of a hornwort sporophyte somewhat complicates this generalization, but for all practical purposes the growth potential for a hornwort is equivalent to that for a moss (Figure 5). The remaining embryophytes are recognized by Kenrick and Crane (1991) as "polysporangiophytes" (Figure 1), because branching of the sporophyte imparts a potential for producing more than one sporangium (e.g., Figure 6).

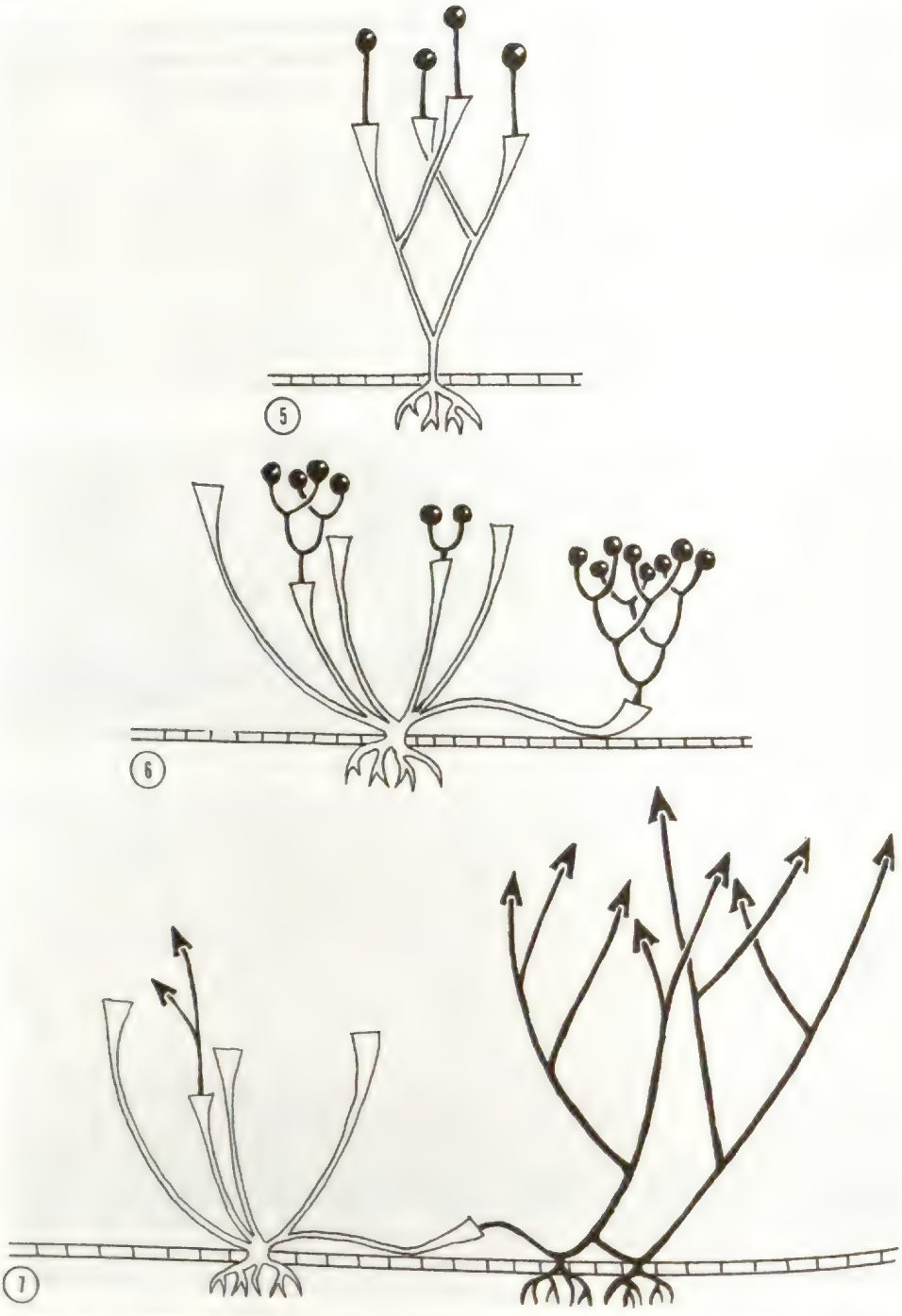
2. Branching Apical or Lateral (Axillary). Among polysporangiophytes, the external form of a sporophyte shoot system can be understood as having resulted from branching that is either apical or lateral. In plants with apical branching, all of the growth of the shoot occurs at the tips of the branches (i.e., periphery of the shoot system; Figures 6–10). Together, these branch tips define the surface of the crown in a tree such as the fossil lycophyte, *Lepidodendron* (Taylor, 1981) and the margin of the "colony" in herbaceous forms such as *Lycopodium digitatum*.

Although there are several types of lateral branching among embryophytes, by far the most common form arises as the result of meristems that are located in the axils of leaves (i.e., true axillary branching). Plants with axillary branching grow at the periphery of the shoot system and also within the system when axillary buds become active. This allows plants with axillary branching to continue to fill out from the center of the crown or the "colony" (Figure 11). Of equal or perhaps even greater importance, axillary branching allows for the repair or replacement of shoots by the process of reiteration (Tomlinson, 1983). Damage to the crown of an individual with reiterative growth need not be permanent or fatal.

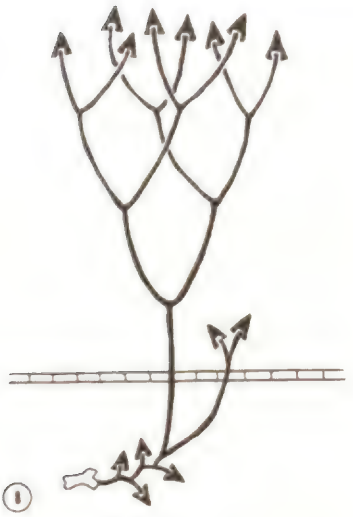
3. Growth Unipolar or Bipolar. In some plants, growth of the sporophytes occurs entirely at the tips of the stems, with the roots arising adventitiously near the base of the stems (e.g., Figure 7). Such sporophytes grow at only one end of the plant body (i.e., one pole) and may be characterized as having "unipolar growth" (e.g., Figure 4). In contrast, many plants have a shoot system that grows at one end (or pole) of the plant body, and a rooting system that grows at the other end (i.e., the opposite pole). Plants of the latter type may be characterized as having "bipolar growth" (e.g., Figures 10, 11).

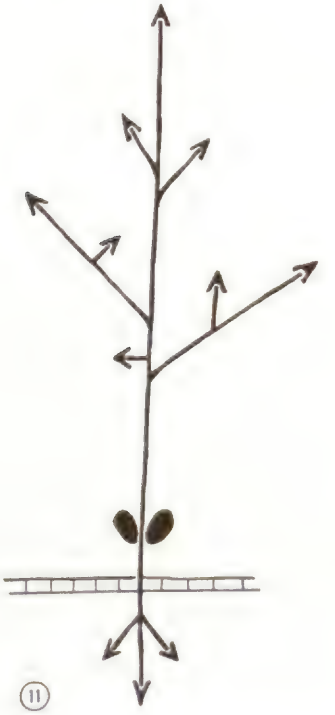
Some plants with the potential for bipolar growth develop unipolar growth as the result of the rooting pole becoming inactive and being replaced by adventitious roots (e.g., grasses). However, in most plants with unipolar growth, no continuously growing rooting pole of the sporophyte develops from the embryo (e.g., *Selaginella selaginoides*, Karrfalt, 1984). Plants with unipolar growth are usually rhizomatous, growing and branching at one end and dying back at the other. Although some plants with unipolar growth do make modest trees (e.g., tree ferns), by and large they are herbs or small shrubs.

In contrast, the embryo of most plants with bipolar growth produces continuously growing and branching meristems at two poles of the plant body (Hallé, Oldeman, and Tomlinson, 1978; Rothwell and Erwin, 1985), one that develops the shoot and another that develops the rooting system of the sporophyte (e.g., Figure 11). The combination of this substantial rooting system and the production of secondary tissue allows plants with bipolar growth to develop into giant trees.



Figures 5–11. Diagrammatic representations of Models of Growth. Gametophyte phase indicated in white with black outline. Sporophyte phase indicated in black. Circles terminating sporophyte represent sporangia and indicate determinate growth. Arrows terminating sporophyte indicate potential for indeterminate growth. Upward pointing arrows depict growth of shoot, whereas downward pointing arrows signify bipolar growth from indeterminate rooting structure. 5. Bryophyte Model individual. 6. Cooksonioid Model individual. 7. Psilotioid Model individual with





terrestrial, *Sciadophyton*-like gametophyte. 8. Psilotioid Model individual with subterranean gametophyte. 9. Selaginelloid Model individual. 10. Isoetoid Model Individual. Note similarity to Selaginelloid Model individual (Figure 9), and origin of rhizomorphic rooting system from modified shoot. 11. Cotyledonoid Model individual. Note potential for growth within crown from axillary branches.

THE ROLE OF BRANCHING IN CHARACTERIZING LAND-PLANT PHYLOGENY

In preliminary phylogenetic analysis that includes the recently developed information about heterogeneity among conducting cells of the most ancient land plants, Kenrick and Crane (1991) helped clarify the relationships among ancient embryophytes and provided additional support for the hypothesis that mosses are the most closely related sister group to what traditionally have been regarded as "vascular plants" (Figure 1; Mishler and Churchill, 1984; Stewart and Rothwell, 1993; Taylor and Taylor, 1993). Employing several criteria, including features of the gametophyte/sporophyte relationship, features of the conducting tissues, and presence/absence of branching, Kenrick and Crane (1991) recognized several grades and clades of land plants. These are a "bryophyte grade," a "protracheophyte grade," and a tracheophyte clade.

The three parameters of branching discussed earlier in this paper allow us to recognize several fundamental modes of branching among embryophytes. When considered in conjunction with major types of embryophyte life cycles, these modes of branching more finely subdivide the polysporangiophytes of Kenrick and Crane (1991) and produce models of growth (Figures 5–11) that help resolve more clearly the relationships among land plants (Figure 1). Character transformations associated with the definitions of these models (Table 1) also reveal synapomorphies for hypothesized clades that have been recognized earlier from other characters (Figure 12; Crane, 1991). The most commonly occurring models are as follows:

Bryophyte Model (Figure 5)

Distinguishing Characters:

1. Individual consists of gametophyte plus sporophyte.
2. Sporophyte is unbranched.
3. Sporophyte has determinate growth (terminal sporangium).
4. Sporophyte has unipolar growth.

Among embryophytes, the sporophytes of liverworts (when present), hornworts, and mosses are characteristically unbranched with a terminal sporangium. Using mosses as an example, we can recognize that the sporophytes of bryophytes occur on branches of the gametophyte. Therefore a bryophyte individual consists of both the gametophyte and sporophyte phases of the life cycle, with all of the branching occurring in the gametophyte phase (Figure 5). The sporophyte is small with determinant growth, whereas the gametophyte theoretically has indeterminate growth and the potential to produce more than one sporophyte.

Mosses, hornworts, and liverworts (when a sporophyte is produced) conform to the Bryophyte Grade. This model differs from all other models of branching by virtue of the fact that the sporophytes do not branch (Figure 12). At the present time it is not certain whether the absence of branching is ancestral among embryophytes (as widely believed), or if the loss of branching potential may be derived in mosses. Fossil specimens interpreted to be a thallose bryophyte with numerous unbranched sporophytes (i.e., *Sporogonites exuberans*, Andrews, 1961) occur in Lower Devonian deposits of Norway. If correctly interpreted, *S. exuberans* demonstrates that land plants of the bryophyte model (Figure 1) existed shortly after the first evidence for polysporangiophytes. Gametophytes of thallose hepatics occur in Upper Devonian sediments (Hueber, 1961), and there is evidence for leafy mosses from the Pennsylvanian (Stewart and Rothwell, 1993). However, the life cycle of the most ancient hepatics and mosses has not yet been demonstrated from fossil evidence.

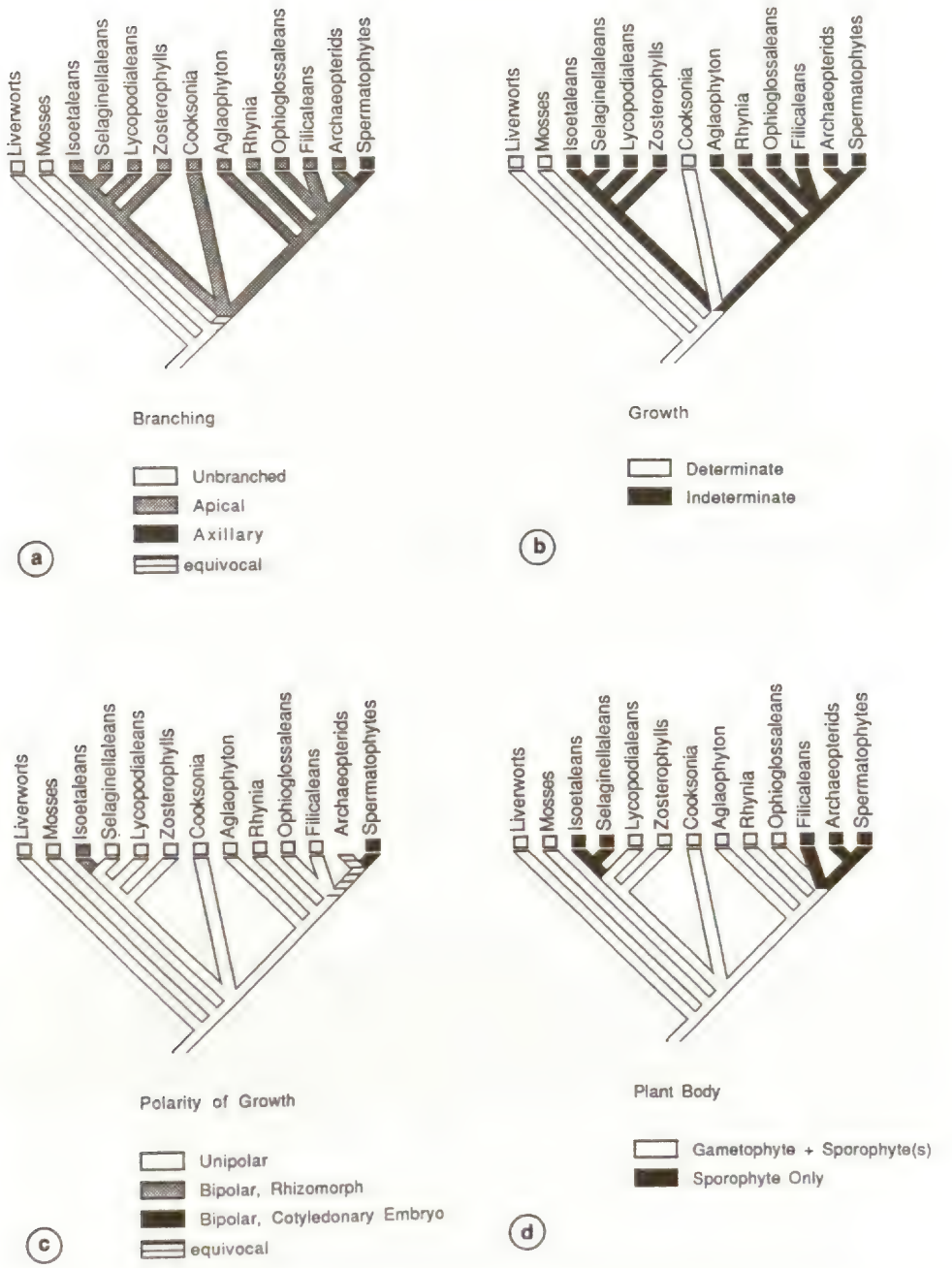


Figure 12. Distribution of characters relating to modes of branching and life cycle as plotted on embryophyte tree (modified from Crane, 1991) in Figure 1. See text for details of character definitions.

Cooksonioid Model (Figure 6)

Distinguishing Characters:

1. Individual consists of gametophyte plus sporophyte.
2. Sporophyte is branched.
3. Branching is terminal.
4. Sporophyte has determinate growth (terminal sporangia).
5. Sporophyte has unipolar growth.

Cooksonioid Model plants are very similar to Bryophyte Model plants with the exception that the sporophyte branches one or more times (Figures 5, 6). A Cooksonioid Model individual consists of both gametophyte and sporophyte phases of the life cycle, where the sporophytes are supported above the ground by the gametophyte. There are no living embryophytes that display Cooksonioid Model branching. Cooksonioid Grade sporophytes probably produced no roots or rhizoids.

Cooksonia (Figure 2) is the smallest, simplest, and most ancient of the Polysporangiophytes (Stewart and Rothwell, 1993). Although classified as a protracheophyte by Kenrick and Crane (1991), the type species of *Cooksonia* (*C. pertoni*) was placed closer to the Rhyniaceae (sensu Kenrick and Crane, 1991) by Edwards, Davies, and Axe (1992), using recently documented characters. Specimens of *Cooksonia* consist of small, dichotomously branched axes with terminal sporangia (Figure 2). Virtually all axes either terminate in a sporangium or are broken off (D. Edwards, pers. comm.). As a result, we may interpret *Cooksonia* to have determinate growth wherein the sporophyte branched one or more times before all of the axes simultaneously produced sporangia. Specimens of *Cooksonia* also are all broken at the proximal end (Figure 2 at arrow), so that potential anchoring or rooting structures are unknown. Nevertheless, the consistently small size of the specimens of *Cooksonia* suggests that the sporophyte probably was not much larger than is represented by the fossils.

Up to the present time, no complete individual of a Cooksonioid Model plant has been discovered. Nevertheless, by combining our knowledge of *Cooksonia* and of the Devonian gametophytes discussed above, one can hypothesize that such an individual consisted of a *Cooksonia*-like sporophyte that was borne in the position of an archegonium on a gametangiophore of a *Sciadophyton*-type gametophyte (Figures 3, 6). The Cooksonioid Model individual may be considered as a candidate for the archetype of primitive land plants. The life cycle of such an individual differs dramatically from the traditional interpretation of ancestral vascular plants (Stewart and Rothwell, 1993), but is somewhat similar to the hypothesis of growth presented by Schweitzer (1983) for *Zosterophyllum*. This emphasizes the difficulties inherent in attempts to classify the most ancient embryophytes as either vascular plants or bryophytes (Remy, 1982). Additional Devonian species that appear to conform to the Cooksonioid Model are *Hedeia corymbosa* (Lang and Cookson, 1935) and *Uskiella spargens* (Shute and Edwards, 1989).

Psilotioid Model (Figures 7, 8)

Distinguishing Characters:

1. Individual consists of gametophyte plus sporophyte.
2. Sporophyte is branched.
3. Branching is predominantly terminal.
4. Sporophyte has potentially indeterminate growth.
5. Sporophyte has unipolar growth.

As with Bryophyte and Cooksonioid Model plants, an individual of the Psilotioid Model consists of both the gametophyte and sporophyte phases of the life cycle (Figures 7, 8). The sporophytes branch as do the Cooksonioids, but not all of the axes terminate in sporangia at the same time. As a result, Psilotioid Model plants have larger sporophytes with the potential for indeterminate growth (Figure 12b). Moreover, the larger Psilotioid Model sporophytes are anchored by rhizoids or adventitious roots, so that they have the potential of existing independently of the gametophyte (Figure 8). *Aglaophyton major* (Edwards, 1986), *Horneophyton lignieri* (Eggert, 1974), and *Rhynia gwynne-vaughani* are examples of Psilotioid Model plants from the Lower Devonian Rhynie Chert. All three species have been convincingly correlated with gametangiophores of the *Sciadophyton*-type (Remy and Hass, 1991; Remy, Gensel, and Hass, 1993).

Among living plants, Psilotioid Model species occur in the Psilopsida (i.e., *Psilotum* and *Tmesipteris*), Lycopodiales (Figure 4), and ferns of the Ophioglossales. Extant Psilotioid Model sporophytes traditionally have been considered to be independent of the gametophyte. This is due, at least in part, to the subterranean location of the gametophytes (Figure 8). However, when living Psilotioid Model species are excavated to show the morphology of the entire plant, they are seen to consist of a basal gametophytic region that produces one or more sporophytes distally (Figure 4). They are, therefore, fundamentally an individual that consists of the gametophyte phase proximally and the sporophyte phase distally. Living Psilotioid Model plants differ from the Devonian Psilotioid Model plants primarily by the smaller size and subterranean position of their gametophytes (Figures 7, 8). By outgroup comparison to bryophytes (Figure 1), indeterminate growth (which distinguishes Psilotioid Model plants from Cooksonioid Model plants) is a derived character within polysporangiophytes (Figure 12b).

Selaginelloid Model (Figure 9)

Distinguishing Characters:

1. Independent gametophyte and sporophyte individuals.
2. Sporophyte is branched.
3. Branching is predominantly terminal.
4. Sporophyte has potentially indeterminate growth.
5. Sporophyte has unipolar growth.

In Selaginelloid Model plants, the gametophyte dies immediately after embryogeny and emergence of the sporeling. Therefore, the sporophyte is obligately independent of the gametophyte beyond the embryonic stage. In plants with this model of growth, the most obvious plant body of an individual consists of only the sporophyte phase. This independence of the sporophyte/gametophyte phases of the life cycle in Selaginelloid Model plants provides the concept upon which the ancestral life cycle of vascular plants traditionally has been based (Figure 9; Gifford and Foster, 1989). Except by comparison to the growth of related living plants, the fossil record provides little direct evidence of the Selaginelloid Model life cycle.

Selaginelloid Model plants differ from Psilotioid Model plants primarily by the ephemeral nature of the gametophyte (Figures 7, 9), which necessitates that the sporophyte live independently beyond the embryonic stage. This feature is characteristic of all heterosporous plants, of *Equisetum*, of marattiaceous ferns, and is most common for filicalean ferns. By outgroup comparison to bryophytes and other pteridophytes, an ephemeral gametophyte phase is considered to be a derived character (Figure 12d). However, the wide distribution of Selaginelloid Model species among only distantly related land plants suggests that this model of growth has arisen independently within several groups (Figure 12d). Paleobotanical

evidence of heterospory suggests that Selaginelloid Model species evolved among barinophytes and stauropterid ferns in the Upper Devonian, among the lycophytes in the Lower Carboniferous, and among heterosporous leptosporangiate ferns in the Lower Cretaceous (Skog and Dilcher, 1992; Rothwell and Stockey, 1993).

Cotyledonoid Model (Figure 11)

Distinguishing Characters:

1. Independent gametophyte and sporophyte individuals.
2. Sporophyte is branched.
3. Branching is lateral (axillary).
4. Sporophyte has potentially indeterminate growth.
5. Sporophyte has bipolar growth from cotyledonary embryo.

Cotyledonary Model growth is the most widely recognized mode of growth for vascular plants. Sporophytes develop from a cotyledonary embryo that produces two embryonic poles of meristematic growth (Figure 11). These meristems are frequently termed the epicotyl and radicle (Raven et al., 1992). Cotyledonary embryos that produce bipolar growth are found only among seed plants. At the present time, we do not know if cotyledonary embryos characterized the progymnospermous sister group (i.e., Archaeopteridales) to seed plants (Figure 1) or if they were derived within primitive seed plants (i.e., stem spermatophytes sensu Rothwell and Serbet, 1994).

Cotyledonary Model plants are also characterized by axillary branching. Hallé et al. (1978) emphasized the significance of this latter feature for facilitating reiteration of damaged sporophytes. Axillary branching is considered to be a synapomorphy of seed plants (Figure 12; Crane, 1985; Doyle and Donoghue, 1986; Rothwell and Serbet, 1994). The earliest evidence for this feature occurs in Lower Carboniferous deposits of Europe (Galtier, 1967). The earliest evidence for plants that produce both axillary branches and cotyledonary embryos, and therefore unequivocally demonstrate Cotyledonoid Model growth, is from Pennsylvanian deposits (Mapes and Rothwell, 1988).

Isoetoid Model (Figure 10)

Distinguishing Characters:

1. Independent gametophyte and sporophyte individuals.
2. Sporophyte is branched.
3. Branching is predominantly apical.
4. Sporophyte has potentially indeterminate growth.
5. Sporophyte has bipolar growth resulting from the production of a rhizomorphic rooting system.

Isoetoid Model plants have terminal branching, potentially indeterminate growth (but see Eggert, 1961), and bipolar growth. As in the seed plants, there is an ascending shoot system and a descending rooting system. However, in Isoetoid Model plants, only a shoot system emerges from the embryo. The rooting system, termed a rhizomorph, is produced by a precocious fork of the shoot apex (Phillips, 1979), one branch of which continues as the shoot and the other branch of which grows downward to form the rhizomorphic rooting system (Figure 10; Stubblefield and Rothwell, 1981; Rothwell and Erwin, 1985). The rhizomorphic rooting system is, therefore, a shoot system that is modified for rooting. As with Cotyledonary Model plants, Isoetoid Model plants can attain the stature of giant trees, but bipolar growth is clearly the result of convergent evolution in the two Models (Figure 12c). The api-

cal branching of Isoetoid Model plants further distinguishes them from Cotyledonoid Model plants (Figure 12a), and significantly restricts their potential for reiteration.

Isoetoid Model growth is found among plants that traditionally have been classified as the Lepidodendrales, Pleuromeiales, and Isoetales (i.e., the "isoetalean clade" of Stewart and Rothwell, 1993; Figure 1). Other than displaying bipolar growth, Isoetoid Model plants have the greatest similarity to Selaginelloid Model plants (Figures 9, 10). The earliest fossil evidence for Isoetoid Model plants occurs in the Upper Devonian (i.e., *Lepidosigillaria*, Rothwell and Erwin, 1985; and possibly also *Eospermatopteris*, Pigg, 1992), and fossil evidence for Isoetoid Model embryos has been documented in Pennsylvanian and Upper Carboniferous deposits (Phillips, 1979; Stubblefield and Rothwell, 1981). By outgroup comparison, bipolar growth is considered to be derived among the lycophytes (Figure 12c; Rothwell and Erwin, 1985), and rooting by a rhizomorph is interpreted to be a synapomorphy for the Isoetalean clade sensu Stewart and Rothwell (1993; Rothwell, 1990; Bateman, DiMichele, and Willard, 1992; Pigg, 1992).

BIOLOGICAL AND PHYLOGENETIC CORRELATES OF BRANCHING

The six models of growth detailed above provide a framework within which to characterize elemental similarities and differences in the growth potentials and life cycles for most embryophytes (Table 1). In varying degrees, these models also are correlated with embryophyte phylogeny (Figures 1, 12) and with the evolutionary ecology of terrestrial colonization.

The Bryophyte and Cooksonioid Models display the simplest modes of growth. They have in common unipolar growth of the sporophyte (Figure 12c), determinate growth of the sporophyte (Figure 12b), and an individual that consists of both the gametophyte and sporophyte phases of the life cycle (Figures 5, 6, 12d). They differ from each other only by the presence or absence of branching in the sporophyte (Table 1; Figure 12a). The distribution of these models of branching among embryophytes (Figure 1) implies strongly that they display the ancestral life cycle for both bryophytes and "tracheophytes" (Figure 12d).

In contrast to Selaginelloid Model plants that require the gametophyte and sporophyte phases of the life cycle to each become established within the environment, Bryophyte and Cooksonioid Model plants require only one establishment event per life cycle. During the initial colonization of land, we envisage that this would have provided a selective advantage in the hostile environments represented by an essentially barren land surface.

At the present time, branching of the sporophyte is the best character for separating bryophytes from "tracheophytes" (Figures 1, 12a), but we have insufficient evidence to demonstrate conclusively whether (1) some bryophytes were derived by suppression of the potential for the sporophyte to branch, (2) polysporangiophytes were derived by obtaining the potential for the sporophyte to branch, or (3) if the ancestors of some bryophytes included both branched and unbranched species. Nevertheless, strong correlations between models of branching and life cycles (i.e., characters plotted on the embryophyte tree in Figure 12) support hypotheses that bryophytes and cooksonioids are closely related (Figure 1).

Psilotioid Model plants differ from Cooksonioid Model species by having the potential for indeterminate growth and the concomitant potential for the sporophyte to be anchored to the ground by rhizoids or roots (Figures 7, 8; Table 1). Distribution of the potential for indeterminate growth among embryophytes (Kenrick and Crane, 1991) indicates that this character is derived in comparison to determinant growth found in Bryophyte and Cooksonioid Model plants (Figure 12b).

Although a Psilotioid Model individual consists of both the gametophyte and sporophyte phases of the life cycle (Figures 7, 8), rooting of the sporophyte imparts a potential for the sporophyte to live independently of the gametophyte. In Selaginelloid Model species,

which differ from Psilotioid Model species by having sporophytes that are obligately independent of the gametophytes beyond embryogeny (Figures 7, 9), this potential for rooting of the sporophyte becomes an essential character for survival. Rooting of the sporophyte also may be a prerequisite for the evolution of a heterosporous life cycle.

Isoetoid Model branching differs from Selaginelloid Model branching by the occurrence of bipolar growth (Figure 12c) which was derived when part of the shoot system became modified for rooting (i.e., a rhizomorph, Rothwell and Erwin, 1985; Figures 9, 10). In terms of growth potential, bipolar growth allows sporophytes to produce larger trees. This potential arborescence was probably a major factor in the ability of lepidodendralean lycophytes to produce the canopy species for tropical forests of the Carboniferous (Phillips, Peppers, and DiMichele, 1985). Bipolar growth from a rhizomorphic rooting system is a synapomorphy for the Isoetales sensu DiMichele and Hueber (DiMichele and Skog, 1992; Figure 12c).

While bipolar growth from the cotyledonary embryo imparts a similar potential to that from a rhizomorphic rooting system (Figure 12c), axillary branching allows for a much greater flexibility in development and for reiteration of the shoot systems of species with Cotyledonary Model branching. It is reasonable to interpret a combination of these two facets of branching as important factors contributing to the rich taxonomic diversity and dominance of Cotyledonary Model plants from the Permian to the present.

The combination of bipolar growth from a cotyledonary embryo and axillary branching is restricted to seed plants (Figures 12a, 12c). Both characters are potential synapomorphies for a clade that includes seed plants and preovulate seed ferns (Rothwell, 1990; Rothwell and Serbet, 1994). However, the distribution of these characters among the extinct species of this clade has not been determined (Serbet and Rothwell, 1992). To help clarify the architectural and systematic significance of bipolar growth among seed plants, we eagerly await the discovery of fossil embryos for progymnosperms, for preovulate seed ferns, and for the most ancient groups of seed plants that produce ovules with a complete integument.

LITERATURE CITED

Andrews, H. N. 1961. Studies in Paleobotany. J. Wiley and Sons, New York.

Bateman, R. M., W. A. DiMichele, and D. A. Willard. 1992. Experimental cladistic analysis of anatomically preserved arborescent lycopsids from the Carboniferous of Euramerica: An essay on paleobotanical phylogenetics. Ann. Missouri Bot. Gard. 79: 500–559.

Crane, P. R. 1985. Phylogenetic analysis of seed plants and the origin of angiosperms. Ann. Missouri Bot. Gard. 72: 716–793.

_____. 1991. The phylogenetic context of microsporogenesis. Pp. 11–42 *in* S. Blackmore and R. B. Knox (eds.), Microspores, Evolution, and Ontogeny. Academic Press, London.

DiMichele, W. A. and J. E. Skog. 1992. The Lycopsida: a symposium. Ann. Missouri Bot. Gard. 79: 447–449.

Doyle, J. A. and M. J. Donoghue. 1986. Seed plant phylogeny and the origin of angiosperms: An experimental cladistic approach. Bot. Rev. (Lancaster) 52: 321–431.

Edwards, D., K. L. Davies, and L. Axe. 1992. A vascular conducting strand in the early land plant *Cooksonia*. Nature 357: 683–685.

_____ and D. S. Edwards. 1986. A reconsideration of the Rhyniophytina Banks. Pp. 199–220 *in* R. A. Spicer and B. A. Thomas (eds.), Systematic and Taxonomic Approaches in Palaeobotany. Oxford Univ. Press, Oxford.

Edwards, D. S. 1986. *Aglaophyton major*, a non-vascular land-plant from the Devonian Rhynie Chert. Bot. J. Linn. Soc. 93: 173–204.

Eggert, D. A. 1961. Ontogeny of the Carboniferous arborescent Lycopsida. Palaeontographica 108B: 43–92.

_____. 1974. The sporangium of *Horneophyton ligneri*. Amer. J. Bot. 61: 405–413.

Galtier, J. 1975. Variabilité anatomique et ramification des tiges de *Calamopitys*. Compt. Rend. Hebd. Seances Acad. Sci., Ser. D. 280: 1967–1970.

Gifford, E. M. and A. S. Foster. 1989. Morphology and Evolution of Vascular Plants. 3rd. ed. W. H. Freeman, New York.

Hallé, F., R. A. A. Oldeman, and P. B. Tomlinson. 1978. Tropical Trees and Forests, an Architectural Analysis. Springer-Verlag, Berlin.

Hemsley, A. R. 1992. Vascular pipe dreams. Nature 357: 641–642.

Hueber, F. M. 1961. *Hepaticites devonicus*, a new fossil liverwort from the Devonian of New York. Ann. Missouri Bot. Gard. 48: 125–132.

_____. 1982. *Taeniocrada dubia* Kr. and W.: its conducting strand and helically strengthened tubes. Abstract. Amer. J. Bot. 69: 58–59.

Karrfalt, E. E. 1984. The comparative and developmental morphology of the root system of *Selaginella selaginoides* (L.) Link. Amer. J. Bot. 68: 244–253.

Kenrick, P. and P. R. Crane. 1991. Water-conducting cells in early fossil land plants: Implications for the early evolution of tracheophytes. Bot. Gaz. 152: 335–356.

_____, D. Edwards, and R. C. Dales. 1991. Novel ultrastructure in water-conducting cells of the Lower Devonian plant *Sennicaulus hippocrepiformis* Edwards. Palaeontology 34: 751–766.

_____, W. Remy, and P. R. Crane. 1991. The structure of water-conducting cells in the enigmatic early land plants *Stockmansella langii* Fairon-Demaret, *Huvenia kleui* Hass et Remy, and *Sciadophyton* sp. Remy et al. 1980. Argum. Palaeobot. 8: 179–191.

Lang, W. H. and I. C. Cookson. 1935. On a flora, including vascular plants associated with *Monograptus*, in rocks of Silurian age, from Victoria, Australia. Philos. Trans., Ser. B. 224: 421–449.

Mapes, G. and G. W. Rothwell. 1988. Diversity among Hamilton conifers. Pp. 225–244 *in* G. Mapes and R. H. Mapes (eds.), Regional Geology and Paleontology of Upper Paleozoic Hamilton Quarry Area in Southeastern Kansas. Kansas Geol. Surv., Guidebook Ser., Lawrence, Vol. 6.

Mishler, B. D. and S. P. Churchill. 1984. A cladistic approach to the phylogeny of the "bryophytes." Brittonia 36: 406–424.

Phillips, T. L. 1979. Reproduction of heterosporous arborescent lycopods in the Mississippian-Pennsylvanian of Euramerica. Rev. Palaeobot. Palynol. 27: 239–289.

_____, R. A. Peppers, and W. A. DiMichele. 1985. Stratigraphic and interregional changes in Pennsylvanian coal-swamp vegetation: environmental inferences. Int. J. Coal Geol. 5: 43–109.

Pigg, K. B. 1992. Evolution of isoetalean lycopsids. Ann. Missouri Bot. Gard. 79: 589–612.

Raven, P. H., R. F. Evert, and S. E. Eichhorn. 1992. Biology of Plants, 5th ed. Worth Pub., New York.

Remy, W. 1982. Lower Devonian gametophytes: Relation to the phylogeny of land plants. Science 215: 1625–1627.

_____, P. G. Gensel, and H. Hass. 1993. The gametophyte generation of some Early Devonian land plants. Int. J. Plant Sci. 154: 35–58.

_____ and H. Hass. 1991. Gametophyton und Sporophyten im Unterdevon-Fakten und Spekulationen. Argum. Palaeobot. 8: 193–223.

_____, _____, and S. Schultka. 1991. Der einzige Vertreter eines unterdevonischen Bauplanes? Courier Forschungsinst. Senckenberg 147: 87–92.

_____ and R. Remy. 1980. *Lyonophyton rhyniensis* nov. gen. et spec., ein Gametophyt aus dem Chert von Rhynie (Unterdevon, Scotland). Argum. Palaeobot. 6: 37–72.

_____, _____, H. Hass, S. Schultka, and F. Franzmeyer. 1980a. Sciadophyton Steinmann—Ein Gametophyt aus dem Siegn. Argum. Palaeobot. 6: 73–94.

_____, S. Schultka, and H. Hass. 1991. *Caliculiphyton blanai* nov. gen., nov. spec., ein Gametophyt aus dem Ems. Argum. Palaeobot. 8: 119–140.

_____, _____, _____, and F. Franzmeyer. 1980b. *Sciadophyton*-Bestande im Seigen des Rheinischen Schiefergebirges als Beleg fur festlandische Bedingungen. Argum. Palaeobot. 6: 95–114.

Rothwell, G. W. 1990. The Lycopsida: an incidence of insouciant evolutionary innovation. Amer. J. Bot. 76: 199.

_____ and D. M. Erwin. 1985. The rhizomorph apex of *Paurodendron*; implications for homologies among the rooting organs of Lycopsida. Amer. J. Bot. 72: 86–98.

_____ and R. Serbet. 1994. Lignophyte phylogeny and the evolution of spermatophytes; a numerical cladistic analysis. Syst. Bot. 19: 443–482.

_____ and R. A. Stockey. 1993. Heterosporous aquatic ferns in the Upper Cretaceous of western North America. Amer. J. Bot. 80: 112.

Schweitzer, H. J. 1983. Die Unterdevonflora des Rheinlandes, 1. Teil. Palaeontographica 189B: 1–138.

Serbet, R. and G. W. Rothwell. 1992. Characterizing the most primitive seed ferns: I. A reconstruction of *Elkinsia polymorpha*. Int. J. Pl. Sci. 153: 602–621.

Shute, C. H. and D. Edwards. 1989. A new rhyniopsid with novel sporangium organization from the Lower Devonian of South Wales. Bot. J. Linn. Soc. 100: 111–137.

Skog, J. E. and D. L. Dilcher. 1992. A new species of *Marsilea* from the Dakota Formation in central Kansas. Amer. J. Bot. 79: 982–988.

Stewart, W. N. and G. W. Rothwell. 1993. Paleobotany and the Evolution of Plants, 2nd ed. Cambridge Univ. Press, Cambridge.

Stubblefield, S. P. and G. W. Rothwell. 1981. Embryogeny and reproductive biology of *Bothrodendrostrobus mundus* (Lycopsida). Amer. J. Bot. 68: 625–634.

Taylor, T. N. 1981. Paleobotany: An Introduction to Fossil Plant Biology. McGraw-Hill, New York.

_____ and E. L. Taylor. 1993. The Biology and Evolution of Fossil Plants. Prentice Hall, Englewood Cliffs, New Jersey.

Tomlinson, P. B. 1983. Tree architecture—new approaches help to define the elusive biological property of tree form. Amer. Sci. 71: 141–150.

Trivett, M. L. C. 1991. Modeling the growth architecture of fossil plants. Ph.D. Dissertation, Ohio University.

ECOLOGICAL AND SYSTEMATIC IMPLICATIONS OF BRANCHING PATTERNS IN BRYOPHYTES

Michael C. F. Proctor[1] and Anthony J. E. Smith[2]

ABSTRACT. The bryophytes embrace several evolutionary lines, which diverged very early from one another and from vascular plants. Branching systems evolved independently in these diverse lines, and (with their generally poikilohydric habit) under different physiological constraints from vascular plants. The major bryophyte groups differ in fundamental details of apical growth and the origin and development of branches. However, the expression of their potentialities in mature branch systems is strongly influenced by environmental factors, especially water-relations and the interception of light and nutrients. Bryophytes are often comparable in size to the thickness of the atmospheric boundary layer, so colony structure—the relation of shoots to one another—is an essential part of their ecophysiological adaptation. The life-forms and branch systems of bryophytes thus reflect interaction between developmental constraints (especially in relation to the position of reproductive structures), internal physiological requirements for translocation and growth, and environmental relationships. Mosses and hepatics show both adaptive radiation and evolutionary convergence in the evolution of branch systems. Such major growth-form characteristics as the acrocarp-pleurocarp distinction in mosses, or the unique structure of *Sphagnum*, may well have evolved first as ecologically based life-form differences, which through stabilizing selection have become a genetically fixed part of the ontogeny of their respective groups. In some instances, as possibly in the leafy hepatics, parts of this process may be under rather simple developmental control. A continuum may be visualized, from characters reflecting basic ontogeny (and significant at high taxonomic levels) to genetically labile features of shoot form and branching varying between or within species—with branching-pattern differences of greater or less taxonomic significance at all levels between these extremes.

INTRODUCTION

Bryophytes have a long evolutionary history, their fossil record reaching back to the Lower Carboniferous (Mosses) and Upper Devonian (Hepatics) (Krassilov and Schuster, 1984). They probably represent several quite separate evolutionary lines, which diverged from the lines that led to the vascular plants very early in the development of plant life on land (Crandall-Stotler, 1980, 1984, 1986; Stebbins and Hill, 1980; Taylor, 1982; Duckett, 1986; Asakawa, 1986). Hence we must see the bryophytes as an essentially independent essay (or rather, several independent essays) in the evolution of branching systems in a terrestrial environment.

As long as an aquatic green plant is near enough the water surface to receive adequate light, all its other needs can generally be met from the bathing medium. On land, water and mineral nutrients may be constantly available below ground; light is only available above the ground surface, where water is present only intermittently and in limited amount, and mineral nutrients may be scarce and hard to acquire. Raven (1977, 1984) has emphasized the importance of the evolution of supracellular transport systems—in particular xylem and

[1]Department of Biological Sciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter, Devon, EX4 4PS, U.K.
[2]School of Biological Sciences, University of Wales Bangor, Bangor, Gwynedd, LL57 2UW, U.K.

phloem—in the evolution of multicellular land plants. The richly branched root systems of vascular plants have evolved in response to requirements for below-ground water and nutrient acquisition, and their above-ground branching systems and leaf-mosaics in response to selection for light interception within constraints imposed by the need to limit water loss. A well-developed vascular system is a prerequisite to connect the two.

Broadly speaking, bryophytes represent an alternative strategy for life on land. They have (essentially, and with minor exceptions) adapted to land life wholly above ground, responding to the intermittent availability of water (where that is a problem) by evolving physiological tolerance of desiccation. Many bryophytes can recover normal metabolism within minutes or hours after drying for long periods at water contents of 10% or less of their dry weight (Proctor, 1990). The shoot systems of bryophytes have thus evolved in response to requirements for water and nutrient acquisition (and retention) as well as light interception. Vascular plants with their well-developed conducting systems are typically orientated along, and exploit, the water-potential gradient from soil to air. Bryophytes tend to occupy a relatively short segment of the water-potential gradient immediately above the ground surface and are often deployed horizontally. Their needs for water conduction are thus less, but also generally driven by relatively small water potential differences; they tend to be met largely by external capillary conduction. Bryophytes suffer important limitations compared with vascular plants. On the other hand, they are freed from some important constraints, especially the requirement for a moisture-providing substratum penetrable by roots, so they can occupy habitats such as bare rock surfaces and the bark of trees, which are inaccessible to vascular plants.

BRANCHING-PATTERN DIFFERENCES BETWEEN THE MAJOR GROUPS OF BRYOPHYTES

A number of the major taxonomic groupings in bryophytes (Table 1) show characteristic differences in the branching patterns that take their origin close to the shoot or thallus apex. Dichotomous or apparently dichotomous branching is common among thalloid bryophytes, where it is an efficient space-filling pattern of growth for radially expanding thalli. The exact nature of this branching—whether from division of a single apical cell or a multicellular apical meristem—has been a matter of controversy (Parihar, 1965; Schuster, 1984a, 1984c). All cases that have been examined critically in recent years point to development from a single apical cell, but this cell may show a very long cell cycle (Crandall-Stotler, 1981; Schofield, 1985; Crum, 1991) and may not be easy to distinguish from neighboring meristematic cells.

In the species that have been investigated, thalli of hornworts (Anthocerotopsida) apparently branch only by true dichotomy, the original apical cell dividing exactly in half to form two equivalent new apical cells (Renzaglia, 1978; Crandall-Stotler, 1981), even though the subsequent growth of the two branches may sometimes be unequal (Schuster, 1984a). The nature of the pinnate or bipinnate branching sometimes seen in such genera as *Dendroceros* and *Megaceros* is not known (Schuster, 1992).

The thalloid liverworts (Hepaticopsida) are varied in their branching patterns, but can at least arguably be regarded as always essentially monopodial in development. In the Marchantiideae, thallus bifurcations often appear very precisely dichotomous, as in the thallus rosettes of *Riccia* (Figure 1b) or the archegoniophores of many of the larger Marchantiaceae (Figure 1c), and have often been considered to originate from truly dichotomous division of the apex. Parihar (1965) and others have regarded the apical meristem as multicellular, consisting of a row of 3–5 or more similar cells, but apical growth is now generally thought

Table 1. An outline of the main groups of bryophytes. In its essentials, this classification is broadly consistent with Parihar (1965), Smith (1978, 1990), Schuster (1984a, 1984c), Vitt (1984), and with much modern fine-structural, cytological, and biochemical evidence; the taxonomic level at which the various groups should be placed is a matter for debate. We regard "bryophytes" as an evolutionary grade, not as a coherent taxon. Hornworts, liverworts, and mosses should probably be treated as three separate divisions, on a level with the Tracheophyta.

ANTHOCEROTOPSIDA (Hornworts)	Thalloid, apical cell usually wedge-shaped, with two lateral and two basal-cutting faces; branching dichotomous.
HEPATICOPSIDA (Liverworts)	
JUNGERMANNIIDEAE Calobryales	Small plants with erect leafy shoots; tetrahedral apical cell with three cutting faces; branches arising from superficial axillary initials, never endogenous.
Jungermanniales (Leafy liverworts)	Leafy, usually dorsiventral; tetrahedral apical cell with three cutting faces; archegonia terminal; branching very varied with both superficial and endogenous origins common.
Metzgeriales (Thalloid liverworts)	Usually thalloid, apical cells various, often lenticular or wedge-shaped with two or more cutting faces; little internal differentiation; archegonia borne dorsally; branching monopodial, often pseudodichotomous.
MARCHANTIIDEAE Marchantiales (Thalloid liverworts)	Thalloid; often with complex internal differentiation; wedge-shaped apical cell with two lateral and two basal cutting faces; apical branching pseudodichotomous, often closely simulating true dichotomy; archegonia borne dorsally.
BRYOPSIDA (Mosses)	Apical cells obovoid segmenting in spiral sequence; branches arising from superficial "axillary" initials, never endogenous.
SPHAGNIDAE (Bog-mosses)	Erect stems of unlimited growth-bearing stem leaves and fascicles of short leafy branches of limited growth; archegonia terminal on short branches, antheridia axillary on leafy branches.
ANDREAEIDAE (Granite mosses)	Stems small, leafy, with terminal archegonia and antheridia; typically forming small cushions on rocks, with limited branching.
BRYIDAE (True mosses)	Stems leafy, erect, or prostrate; archegonia and antheridia terminal on main shoots or short laterals; details of branching very varied.

to be from a single wedge-shaped apical cell with four cutting faces, two lateral and two basal (Crandall-Stotler, 1981; Schuster 1984a; Crum, 1991). Schuster (1992) sees the branching as pseudodichotomous, with new apical cells formed exceedingly close to the apex, and considers that true dichotomy has never been convincingly demonstrated. It is not uncommon for one of the two branches at a fork to be stronger than the other, as in *Conocephalum*. However, as Schuster comments, the precise nature of the apical meristem and branching of Marchantiales among these possibilities is almost a matter of semantics.

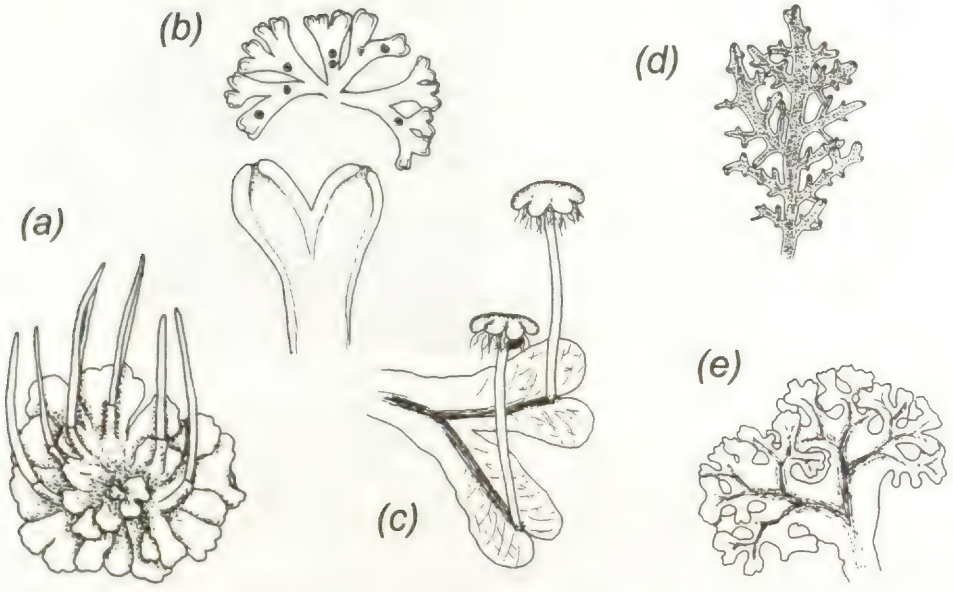


Figure 1. Hornworts and thalloid liverworts. (a) *Phaeoceros laevis* (Anthocerotopsida), thallus with sporophytes; (b) *Riccia glauca* (Hepaticopsida: Marchantiales), thallus rosette with embedded sporophytes and thallus apex enlarged; (c) *Dumortiera hirsuta* (Hepaticopsida: Marchantiales), thallus with two archegoniophores; (d) *Riccardia multifida* (Hepaticopsida: Metzgeriales), monopodially branched thallus; (e) *Pellia endiviifolia* (Hepaticopsida: Metzgeriales), autumn growth showing pseudodichotomous branching. From Smith (1990).

The Metzgeriales (Jungermanniideae) vary greatly in their apical structure as in other characters; their apical cells are most often lenticular, hemidiscoid, or wedge-shaped with two or four cutting faces, but apical cells with three cutting faces occur, for instance, in *Petalophyllum*. Throughout the Metzgeriales, branching is monopodial, the formation of a new branch apical cell from a lateral derivative of the parent apical cell being delayed by at least a few cell divisions. The effect of this is obvious in the branching of such genera as *Aneura* and *Riccardia* (Figure 1d), or *Apometzgeria pubescens*, but the regular bifurcated branching patterns of the kind seen so strikingly in the aerial shoots of *Hymenophyton*, some *Metzgeria* species, and the autumn growth of *Pellia endiviifolia* (Figure 1e) is also essentially monopodial in development (Renzaglia, 1982); the bifurcation is pseudodichotomous.

Shoots of the remaining bryophytes develop from a single apical cell, tetrahedral with three cutting faces in the Calobryales and Jungermanniales, generally more elongated and cutting off derivatives spirally in mosses (Figures 2, 5; Frey, 1981; Berthier, 1972; Crandall-Stotler, 1980; Schofield, 1985).

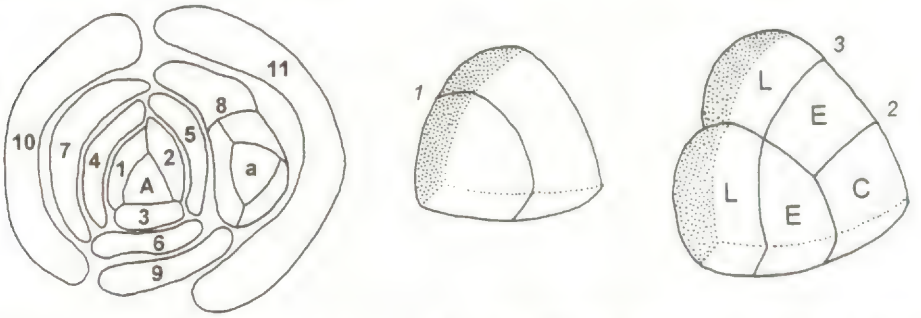


Figure 2. Apical development of leafy liverworts. (Left) Simplified transverse section of apex of *Bazzania* (Hepaticopsida: Jungermanniales), showing the apical cell (A) with three cutting faces, and the strictly 1/3 phyllotaxy, with two rows of larger lateral leaves and a ventral row of smaller underleaves. The segments are numbered from the apex downwards to correspond with Figure 5. The section shows the origin of a branch (apical cell a) replacing the ventral half of a lateral leaf. (Right) Diagrams showing the early segmentations of a derivative from the apical cell. L = foliar initial, E = initial giving rise to stem epidermis, C = initial giving rise to central parenchyma of stem. After Crandall-Stotler (1980).

In the leafy liverworts (Jungermanniideae: Jungermanniales), the apical cell is nearly tetrahedral and the three cutting faces at least potentially produce three rows of leaves. Typically the stems are dorsiventral, with the row of leaves on the ventral side usually smaller than the rest, developing as distinctive "underleaves," or even absent altogether. Archegonia are borne terminally, so the branching pattern is sympodial. Evans (1912) showed that most branches in leafy liverworts (so-called "terminal branches") are formed from half of a lateral (rarely ventral) segment from the apical cell, the other half-segment forming an imperfect leaf (Figure 2). Evans distinguished three basic patterns of branching of this kind; in a fourth (*Radula/Lejeunea*) type, formation of the branch apex from a lateral segment is delayed, so the branch comes to lie close to the base of a complete leaf. From their origin, leafy liverwort branch systems tend to be complanate, reflecting the bilateral symmetry of the stem structures from which they develop. Further research, in recent decades particularly the work of Crandall (1969; see also summary in Crandall-Stotler, 1972) and Schuster (1966, 1984a, 1984b), has demonstrated the diversity in detail and the taxonomic significance of liverwort branching types. In addition to the terminal branches, which originate from superficial cells, some leafy liverwort genera produce endogenous branches that develop from cells at deeper levels within the stem. The variations in branching type stand alongside such features as presence or absence of a ventral row of leaves (underleaves), and details of the form and insertion of the leaves, as apparently fundamental morphological characters of the orders and genera of leafy liverworts.

However, as Basile (1967, 1970, 1979, 1990) has shown, treatment with hydroxy-L-proline or 2-2′dipyridyl can produce a remarkable range of phenovariations in the growth of leafy liverworts, including the development of underleaves in genera in which these are normally lacking. Some of these phenovariations result in formation of leaves and shoots mimicking those of widely different genera or families (Figure 3). These effects appear to result from interference with the formation and morphogenetic action of hydroxyproline-containing cell-wall proteins (Basile, 1990; Basile and Basile, 1990). The experimental evidence sug-

gests that much apparently stable and taxonomically fundamental variation of leaf-form and branching in leafy liverworts results from selective suppression of different elements of a common developmental plan, and that it may be under rather simple morphogenetic control (Stebbins and Basile, 1986).

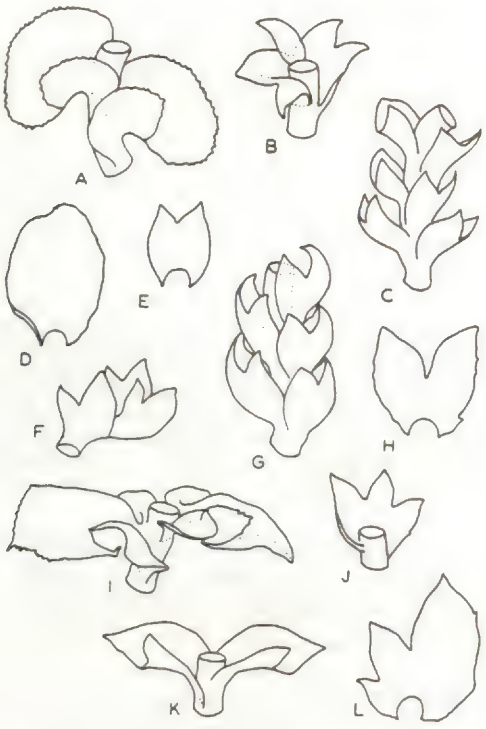


Figure 3. Drawings to show the range of variation in leaf shape resulting from the addition of L-hydroxyproline or 2,2′-dipyridyl to *Scapania nemorosa* gametophytes in axenic culture. The lateral leaves A are characteristic for *S. nemorosa*. Most of the leaves illustrated are atypical of the genus (*Scapania*) and family (Scapaniaceae), but phenocopy those of species belonging to other families in the Jungermanniales. The same treatments produce changes in branch type which cut widely across taxonomic groupings, though they are less striking than the leaf phenovariations to the non-specialist. (From Basile [1974] with permission.)

The mosses (Bryopsida) differ in very fundamental respects from the liverworts. In addition to the true mosses (Bryidae), which are the largest group of bryophytes, probably with around 15,000 species, two small groups, Sphagnidae and Andreaeidae, which probably have very long independent evolutionary histories, are traditionally placed here.

Sphagnum (Sphagnidae) is an isolated genus not closely related to the other mosses (Daniels and Eddy, 1990), though a connection with the Dicranales (Bryideae) has been suggested (Gams, 1959; Berthier, 1972). Characteristic features of the genus include a unique branching pattern. The sparingly branched main stems are orthotropic and potentially of unlimited growth. They develop from an apical cell that cuts off derivatives in a 2/5 spiral sequence. Each division of this corresponds to the formation of one stem leaf. Clusters ("fascicles") of 1–8 or more short branches of limited growth develop in association with every fourth leaf, so these fascicles are themselves arranged in a reverse 2/5 "cladotaxy" (Figure 4; Parihar, 1965; Berthier, 1972; Hill, 1978). The branches are densely leafy, with the leaves in a 2/5 phyllotaxy and often obviously five-ranked. They form the photosynthetic tis-

sue of the plant and are usually differentiated into slender "descending branches" forming a wick round the stem, and more robust and deeply colored "spreading branches." The branch leaves are differentiated into a meshwork of interconnecting living photosynthetic cells, with empty, dead, porose "hyaline cells" in the interstices. The whole structure provides capillary pathways to maintain a constant supply of water to the photosynthetic cells as long as the water table is not too far below the surface; at the same time it provides a large proportion of air- or water-filled voids which provide for gas exchange and can accommodate large variations in water content with minimal change in the level of the water table (Clymo and Hayward, 1982; Ingram, 1984). There are quantitative differences between different species of *Sphagnum* in the number and type of branches in the fascicles.

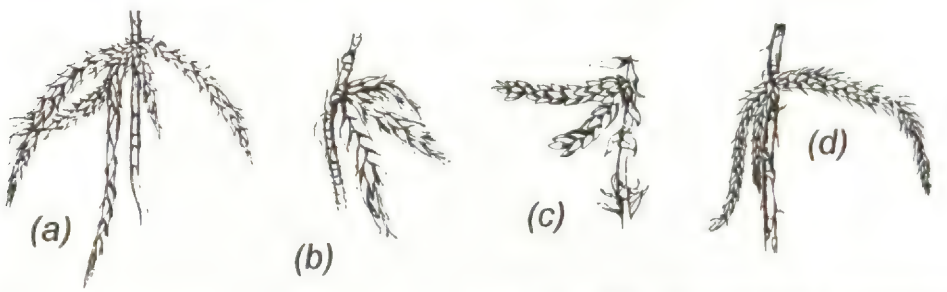


Figure 4. The branching structure of *Sphagnum*; part of stem with a fascicle of branches. (a) *S. quinquefarium*, (b) *S. auriculatum*, (c) *S. tenellum*, (d) *S. flexuosum*. From Smith (1978).

Andreaea is the main genus of another small isolated group ("granite mosses," Andreaeidae). These plants grow in small blackish cushions or patches on acid rocks on mountains and in cold or cool-temperate regions. They resemble small cushion-forming true mosses in the form of their shoots. These grow from a spirally-segmenting apical cell and bear archegonia terminally. Branching is sympodial from initials of superficial origin, but less regular than in many of the acrocarpous Bryidae considered below.

In the true mosses (Bryidae), the apical cell is generally more elongated than in the liverworts, often polygonal in transverse section, and the cutting faces typically follow a spiral sequence (Frey, 1970, 1981; Berthier, 1972; Crandall-Stotler, 1980). The leafy shoots usually show a 2/5 or 3/8 spiral phyllotaxy. Branches develop from superficial "axillary" initials; morphologically, they arise below the leaves (Figure 5), not strictly in the axil of a subtending leaf as in vascular plants (Berthier, 1972; Frey, 1970, 1981; Schofield and Hébant, 1984). Although a branch-initial is formed in association with every leaf, many or most remain dormant, only a proportion developing to form growing points. A common pattern is for three out of four successive axils to remain "empty" (as in *Sphagnum*), one in four developing into a bud that may grow on into a branch, or remain suppressed more or less indefinitely. However, the cladotaxies of mosses are diverse, and larger (or occasionally smaller) numbers of empty axils may intervene between successive potential branches. Sometimes branches are produced adventitiously from empty axils, as with the axillary propagules of *Pohlia* and *Isopterygium elegans* and the axillary antheridial shoots of some Fissidentaceae (Berthier, 1972). The juvenile, usually filamentous, protonema stage is particularly prominent in the Bryidae. It is often important in vegetative increase, either following establishment from spores, or through the development of secondary protonema from gametophyte fragments. Amongst the larger Polytrichales, thick rhizoid strands may simulate rhizome-like branching underground axes.

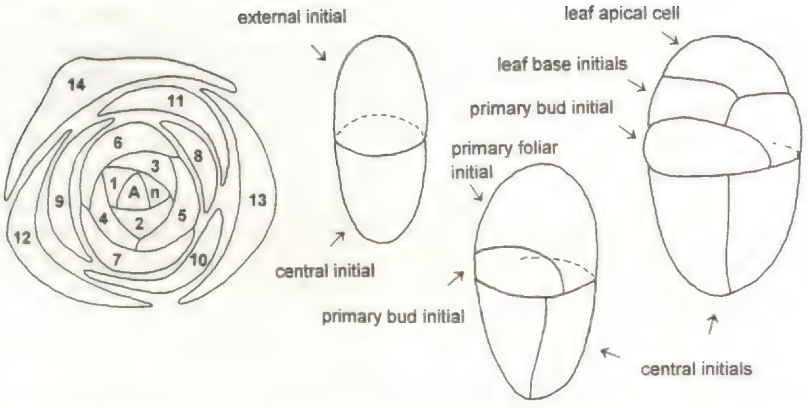


Figure 5. Apical development of mosses (Bryopsida). (Left) Simplified transverse section of the apex of *Mnium hornum* (Bryidae: Bryales), showing the oblique segmentation pattern of the apical cell (A) and the 3/8 phyllotaxy of the leaves. The most recent derivative is marked n, with earlier derivatives numbered downwards in sequence. (Right) Diagrams showing the early divisions of a derivative from the apical cell. After Berthier (1972).

The true mosses can be divided into two major branching types (Figure 6; Meusel, 1935; Walther, 1983). In **acrocarpous** mosses, the shoots, usually orthotropic, are of limited growth, the apex ultimately giving rise to an antheridial or archegonial group. Growth is sympodial, continued by branches (innovations) either at the base, or immediately below the apex. Branches in other positions are rare; notable exceptions are the axillary gemmae of some *Bryum* and *Pohlia* species (Lewis and Smith, 1977, 1978; Smith and Whitehouse, 1978). There is a good deal of variation in detail in the branching patterns of acrocarpous mosses; some broad systematic correlations reflecting developmental anatomy are summarized by Berthier (1972). In perennial Bryaceae, Pottiaceae, and other families, innovations often show an annual periodicity (Hagerup, 1935; Lackner, 1939; Jendralski, 1955); innovations formed below antheridial apices may reflect the removal of apical dominance (Berthier, 1972). Annual Funariaceae typically produce an antheridial primary shoot, with an archegonial shoot formed as an innovation from near its base; increase of shoot number in these plants is by new growth from the protonema. Splachnaceae probably follow a similar pattern initially but later produce repeated innovations, both in the annual *Splachnum* species, where they bring about much of the vegetative increase of the clumps, and in other, perennial members of the family (Meusel, 1935).

Pleurocarpous mosses have shoots of potentially unlimited (monopodial) growth and are commonly plagiotropic. Antheridial and archegonial apices are formed on short unbranched laterals, and these along with vegetative branches of limited growth may develop throughout the year's growth segment. Pleurocarpous mosses are often richly branched, their shoot systems are often complanate, and many species show beautiful plumed and fern-like forms. They are among the most abundant and conspicuous mosses of grasslands and forests.

Several 19th century systematists, including Schimper and Limpricht, followed Bridel (1826–1827) in regarding the acrocarp-pleurocarp distinction as a primary taxonomic division within the mosses (Vitt, 1984). It is now clear that pleurocarpy must have arisen independently a number of times in unrelated lines. The work of Berthier (1972) emphasizes features of branch development common throughout the Bryidae, but shows too, for instance, that acrocarps often develop rhizoid initials (and thus stem tomentum) from tissues of the "empty" axils, whereas the corresponding development of paraphyllia (outgrowths of leaf-like tissue between the leaves) tends to be a pleurocarp feature. In recent classifications,

greater taxonomic weight is placed on other characters, including, for instance, the structure of the peristome, and other anatomical details of the gametophyte and sporophyte. However, it remains broadly true that the families and higher units recognized in modern classifications are either uniformly (or at least mainly) acrocarpous (e.g., Polytrichaceae, Dicranaceae, Pottiaceae, Grimmiaceae, Bryaceae) or uniformly pleurocarpous (families in Suborders Hypnineae, Leucodontinineae and Hookeriineae, sensu Vitt, 1984). Some transitional states between acrocarpy and pleurocarpy are considered later in this paper.

BRYOPHYTE GROWTH-FORMS AND LIFE-FORMS; ECOLOGICAL ADAPTATION

The detailed realization of bryophyte branching patterns in the field shows clear relationships with habitat. Of three useful related concepts, growth-form is essentially morphological and life-form essentially ecological (Gimingham and Robertson, 1950)—while life-strategy emphasizes temporal, life-cycle, and functional dimensions of ecological adaptation mostly outside the scope of this paper (During, 1979, 1992; Longton and Schuster, 1984; Grime, Rincon, and Wickerson, 1990). A number of life-form schemes have been devised for bryophytes. Probably the most widely used and quoted is that proposed by Gimingham and Robertson (1950), and subsequently modified and expanded by Gimingham and Birse (1957). However, this was devised primarily for the British moss flora, and omits various life-forms important in other parts of the world, which are included in the more comprehensive schemes of Horikawa and Ando (1952), Mägdefrau (Table 2; 1969, 1982), and Richards (1984).

Cushion life-forms illustrate some basic adaptive features that lend themselves to measurement and experiment. The capillary spaces within the cushion provide for storage of water after rain; the surface of the cushion is the site of water-loss by evaporation. The rate of water-loss is determined by the saturation deficit of the surrounding air, the diffusion-resistance to water-vapor transfer in the laminar boundary layer around the bryophyte, and the availability of energy to supply the latent heat of evaporation (Proctor, 1982, 1990). If the first and third of these remain constant, the rate of water-loss depends on boundary-layer thickness, which is in turn dependent on the size and surface character of the bryophyte cushion and on windspeed. At windspeeds around 1 m/s, the boundary-layer thickness around a moss cushion is likely to be a few hundred micrometers, that is, on a similar scale to the surface irregularities of the cushion. Wind-tunnel measurements show that at very low windspeeds a moss cushion behaves as a smooth simple object; water loss increases approximately as the square-root of the windspeed, reflecting the corresponding inverse decrease in boundary-layer thickness (Proctor, 1981). Beyond a certain critical point, evaporation rises more rapidly with windspeed; the rougher the cushion surface, the lower the windspeed at which this occurs. From simple textbook aerodynamic theory it is tempting to regard this as the point at which the moss cushion begins to generate turbulence in the airflow. That may in part be so, but a moss cushion generates some turbulence in its wake even at low windspeeds, and the abrupt increase in evaporation at a critical windspeed may also reflect the fractal nature of the cushion surface area (Williamson and Lawton, 1991) measured by a boundary-layer of progressively decreasing thickness.

This gives us a theoretical basis from which we can understand why a small cushion life-form is adaptive in open exposed situations on rock or bark, and this can be tested by experiment. In a population of *Grimmia pulvinata* studied in Devon, U.K. (M. C. F. Proctor, unpublished data), moss cushions a few centimeters in diameter stored the equivalent of about 2 mm of rainfall when saturated. Rates of water-loss accorded reasonably well with predictions from meteorological data; the moss cushions dried out within an hour or two in fine

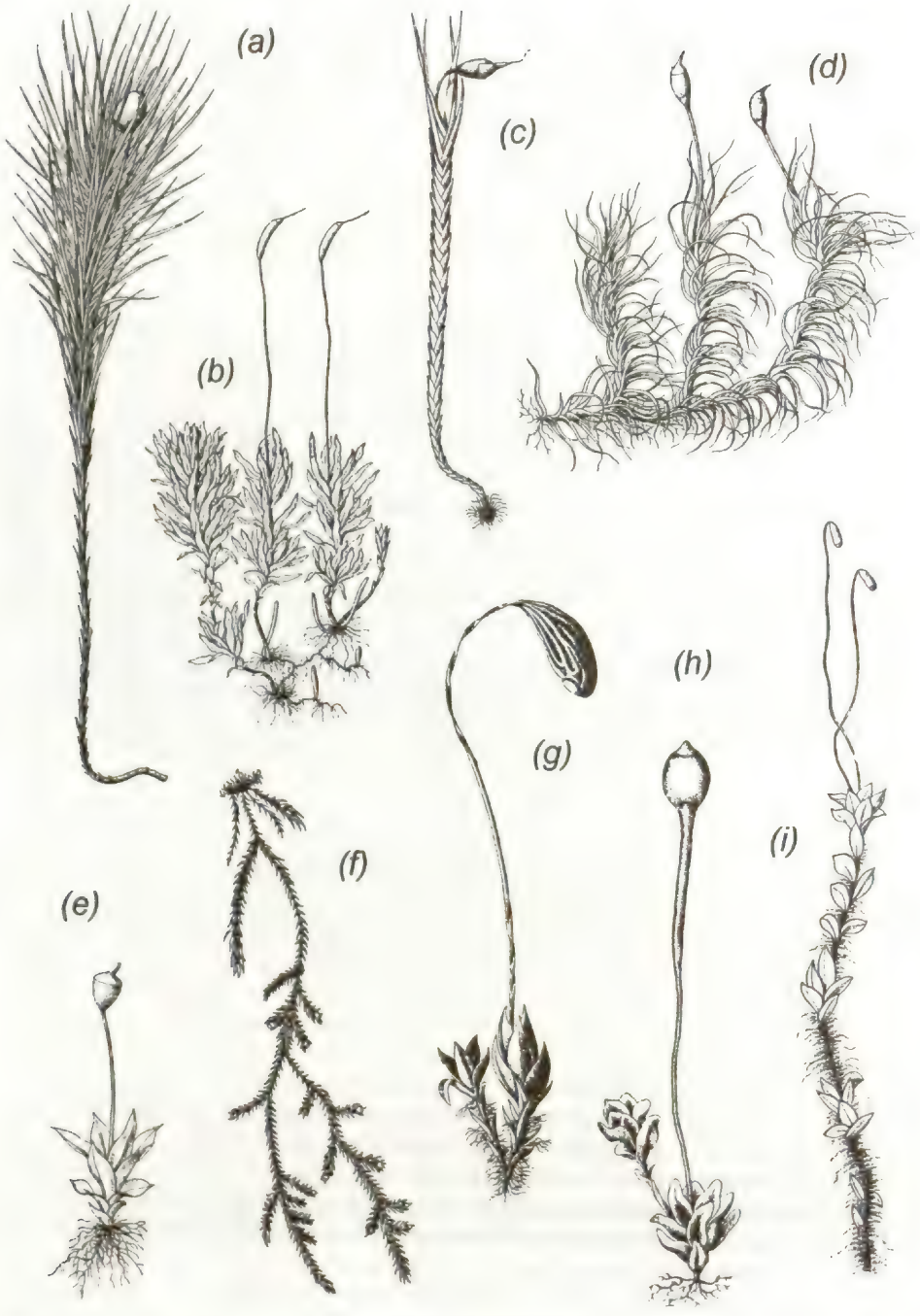
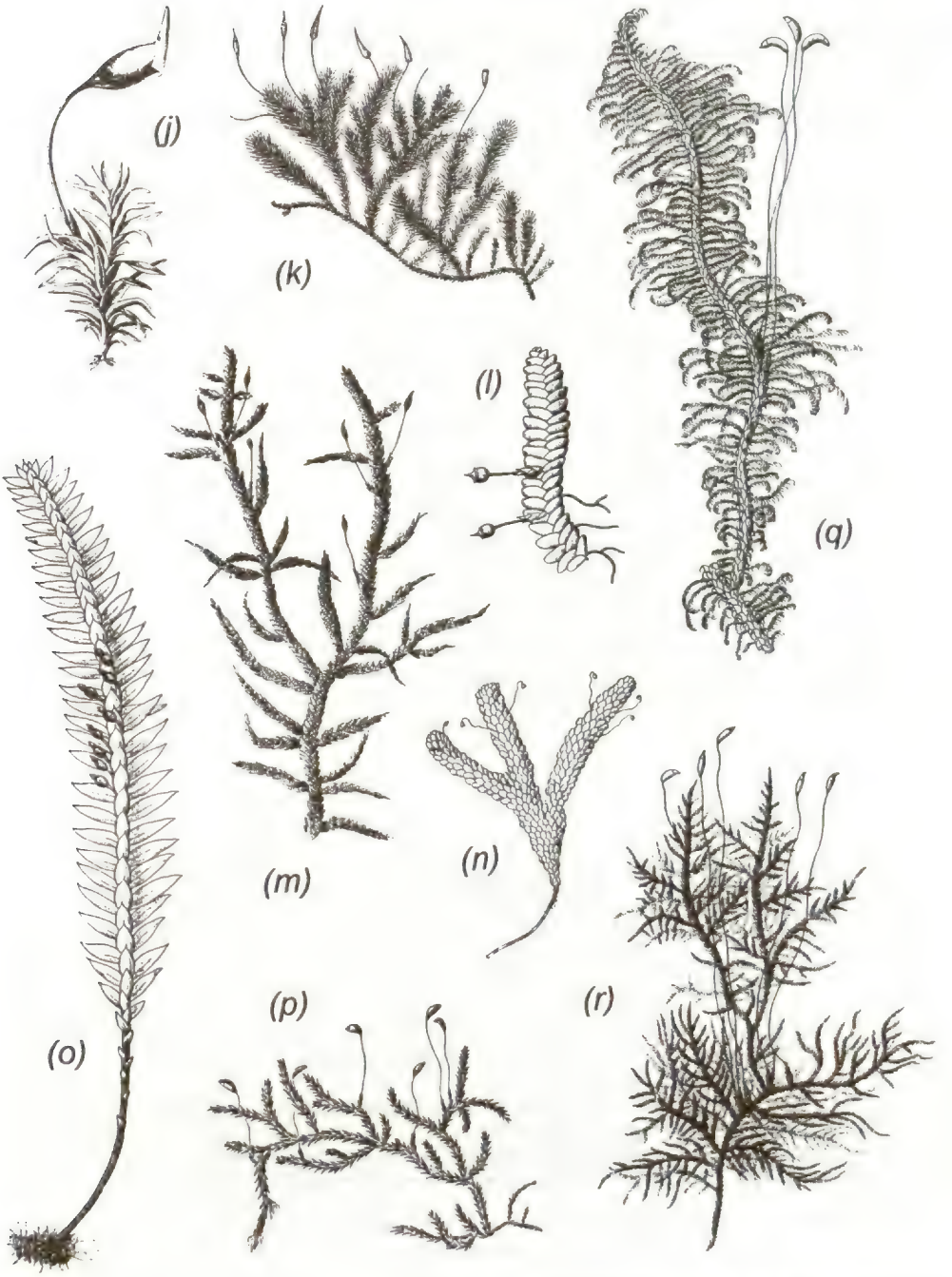


Figure 6. Acrocarpous and pleurocarpous mosses. (a–k) **Acrocarpous genera**. (a) *Dawsonia superba* (Dawsoniaceae) × 0.9; (b) *Atrichum undulatum* (Polytrichaceae) × 1; (c) *Bryoxiphium norvegicum* (Bryoxiphiaceae); (d) *Arctoa fulvella* (Dicranaceae) × 4.8; (e) *Pottia truncata* (Pottiaceae) × 7.6; (f) *Cinclidotus fontinaloides* (Pottiaceae) × 1; (g) *Funaria hygrometrica* (Funariaceae) × 2.8; (h) *Oedipodium griffithianum* (Oedipodiaceae) × 7.6; (i) *Cinclidium stygium* (Mniaceae) × 1; (j) *Zygodon viridissimus* (Orthotrichaceae) × 6.7;



(k) *Macromitrium orthophyllum* (Orthotrichaceae) × 1. (l–r) **Pleurocarpous genera**. (l) *Wildia solmsiellacea* (Erpodiaceae) × 3.3; (m) *Neckera crispa* (Neckeraceae) × 0.7; (n) *Distichophyllum spathulatum* (Hookeriaceae) × 0.6; (o) *Cyathophorum bulbosum* (Hypopterygiaceae) × 0.9; (p) *Brachythecium salebrosum* (Brachytheciaceae) × 0.7; (q) *Ptilium cristacastrensis* (Hypnaceae) × 0.9; (r) *Hylocomium splendens* (Hylocomiaceae) × 0.9. (g), (j), (m,) and (p) from Limpricht (1890–1904), others from Brotherus (1909).

sunny weather in summer, but often remained continuously moist for long periods in mild unsettled weather in the low radiation conditions of autumn and early winter (Figure 7). Measurements showed that most growth took place between September and January (Figure 8).

Table 2. Life-forms of bryophytes: a summary of the classifications proposed by Gimingham and Birse (1957) and Mägdefrau (1982). For further details and examples see the original accounts.

A. Gimingham and Birse (1957).
I. Cushions: Systems with shoots radiating from a central point. 1. Large cushions. Normally > 5 cm diam. (*Leucobryum*). 2. Small cushions. Normally < 5 cm diam. (*Grimmia, Ulota*).
II. Turfs: Systems with parallel upright shoots. 1. Tall turfs (> 2 cm) with erect branches (*Dicranum majus, Tortula ruraliformis*). 2. Tall turfs (> 2 cm) with divergent branches of limited growth (*Sphagnum, Calliergon giganteum*). 3. Short turfs (< 2 cm) (*Ceratodon purpureus, Barbula convoluta*). 4. Open turfs (*Pogonatum aloides, Diphyscium foliosum*).
III. Canopy formers: Dendroid (*Climacium dendroides*).
IV. Mats: Main shoots creeping over substratum, often with rhizoids. 1. Rough mats (*Homalothecium sericeum, Brachythecium rivulare*). 2. Smooth mats (*Hypnum cupressiforme, Frullania dilatata*). 3. Thread-like forms (many species when growing amongst other vegetation). 4. Thalloid mats (*Conocephalum conicum, Pellia epiphylla*).
V. Wefts: Systems of straggling, overlapping and intertwining shoots, not firmly attached to the substratum (*Thuidium, Pseudoscleropodium purum, Rhytidiadelphus loreus, Hylocomium splendens*).

B. Mägdefrau (1982).
(I) Annuals: without regenerative shoots (*Diphyscium, Phascum*, many Funariaceae, *Riccia*).
(ii) Short turfs: c. 1 cm high (*Trichostomum, Barbula, Ceratodon, Marsupella*).
(iii) Tall turfs: (*Dicranum, Polytrichum, Herberta*).
(iv) Cushions: (*Andreaea, Grimmia, Amphidium, Plagiopus*).
(v) Mats: (*Homalothecium, Radula*, most thalloid liverworts).
(vi) Wefts: (many Brachytheciaceae, Hypnaceae, Hylocomiaceae, *Thuidium*).
(vii) Pendants: main shoots hanging from branches and twigs of trees (many Meteoriaceae and Phyllogoniaceae, *Frullania atrata, Macrolejeunea pallescens*).
(viii) Tails: shoots standing out from surfaces of trees or rocks, radial (*Leucodon, Spiridens, Schistochila*).
(ix) Fans: shoots usually flattened, standing out ± horizontally from ± vertical trees, rocks, etc. (Neckeraceae, Pterobryaceae, *Bryopteris*).
(x) Dendroids: (*Climacium, Hypnodendron, Dendroligotrichum, Hymenophyton*).

Because the area for evaporation varies as the square, but the capacity for water storage as the cube, of the linear dimension, initial establishment is a critical time in the life of the cushion, as the experiments of Alpert (1988) with *Grimmia laevigata* illustrate. More open larger cushions or tall turfs are only viable in the shade and shelter provided by forest or other tall vascular-plant cover, or where water is plentiful. Here the balance of selective pressures will move towards light and nutrient acquisition in competition with other bryophytes and with the smaller vascular plants (Proctor, 1984, 1990).

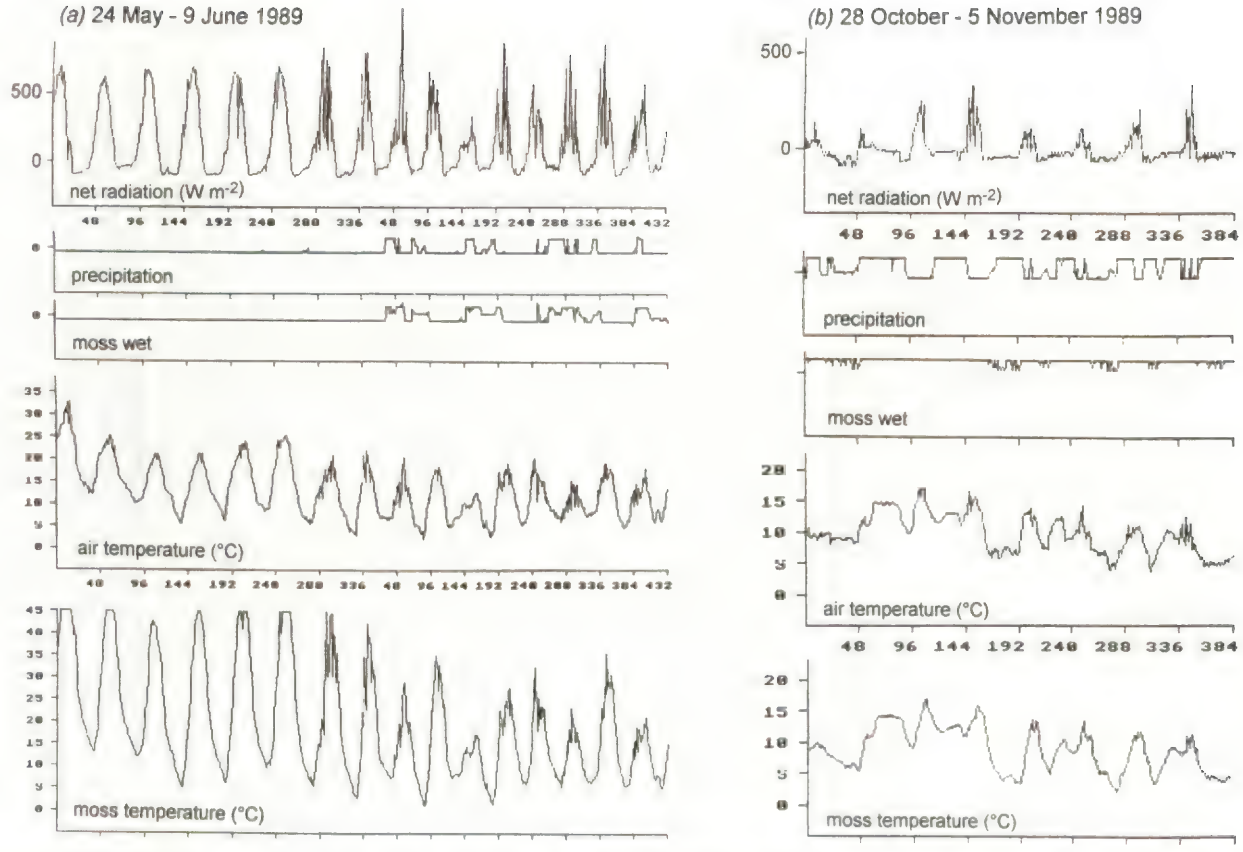


Figure 7. Data-logger output for periods in May–June and October–November 1989, from a population of *Grimmia pulvinata* on a wall-top near Morchard Bishop, Devon, U.K. The line "precipitation" is from a 1-cm-long probe consisting of two parallel platinum wires separated by a figure-eight winding of polyester thread, in a circuit adjusted to give maximal response when wet and zero when dry, exposed above the wall. The line "moss wet" is from a similar probe inserted in a moss cushion. Note the small effect of water storage in the moss cushion in the high-radiation conditions of early summer, compared with almost continuously moist state of the moss with the much lower radiation income in autumn.

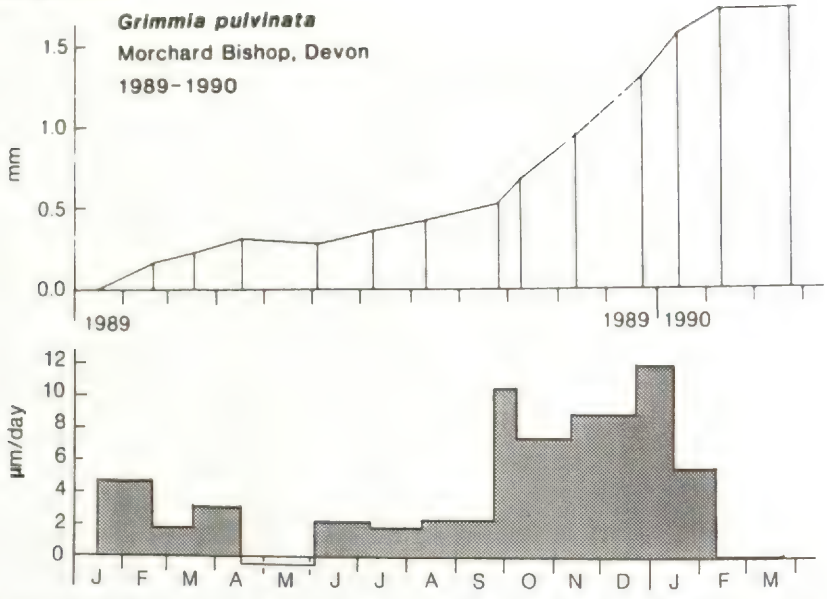


Figure 8. Seasonal growth of *Grimmia pulvinata*, January 1989–March 1990. Morchard Bishop, Devon, U.K. Means of measurements with a miniature pin-frame from five individual cushions. Growth is most rapid in the autumn, with favorable temperature, high rainfall, and low evaporation. Cold probably often limits growth in late winter. Spring and early summer have the lowest rainfall, and high radiation-income favors evaporation, so moss growth in dry habitats is minimal during this period.

Mat life-forms occur mainly on rock and bark in more sheltered situations than small cushions, and weft life-forms are particularly characteristic of the forest floor and of dwarf-shrub and herbaceous communities in which the vascular-plant cover is not too closed. These typically pleurocarpous shoot-systems are well adapted to the interception of light coming largely from overhead (compare the vascular plant canopies considered by Monsi, Uchijima, and Oikawa, 1973; Horn, 1971), and the interception of nutrients from throughfall (and dry deposition). One of the earlier studies to demonstrate this was the work of Tamm (1953) on *Hylocomium splendens* in coniferous forest in Sweden, but the capture and retention of nutrients by pleurocarpous mosses (and other bryophytes) have been confirmed and our understanding of the processes involved greatly expanded by Streeter (1965), Hoffman (1972), Rieley, Richards, and Bebbington (1979), Brown (1982), Bates (1987, 1990), Bates and Farmer (1990), and others (see Brown and Bates, 1990 for references).

In general, closely pinnate complanate branching systems forming a more or less complete ground cover (e.g., *Hylocomium splendens, Thuidium* spp., *Pseudoscleropodium purum, Ptilium crista-castrensis*) are characteristic of communities on nutrient-limited soils, or in shade under forest canopies where the competition is for nutrients with the tree roots rather than for light with the field layer. Open wefts and thread-like forms are more characteristic of nutrient-rich situations with a denser vascular-plant cover (e.g., *Brachythecium rutabulum* and *Lophocolea bidentata* in grassland). Their form may in part be a response to shade and the faster turnover of space among the bases of the vascular plants, but as Rincon (1988) has indicated, they may also reflect a more active pattern of foraging for nutrients from the litter of the vascular plants.

The bryophytes covering the twigs and upper branches of moss-rich forests in both temperate and tropical regions must receive much of their water by interception of rain. But at least some moisture will come from cloudwater or "occult precipitation"—airborne water

droplets caught by impaction that would not be caught by a conventional rain gauge. This can be a major source of water and solutes to surfaces exposed in regions where cloud and mist are frequent (Monteith and Unsworth, 1990). The narrower the surface, the more efficiently it will trap droplets. At windspeeds of a few meters per second, the impaction efficiency for 20 μm water droplets is around 70–90% for a cylindrical obstruction 1 mm in diameter, but only around 10–20% for a 10 mm obstruction such as a twig and lower again for a smooth tree-trunk. In the cloud forests of tropical mountains and in some humid temperate forests, pendant life-forms (Figure 9) are common (Herzog, 1926; Richards, 1984). These undoubtedly function as particularly effective cloudwater collectors. Cloudwater is likely to be a substantial source of moisture also to species of "fan" or "tail" life-forms (Mägdefrau, 1982). A general feature of cloudwater droplets is that they tend to accumulate solutes present in the atmosphere—an important factor in the nutrition of cloudwater-dependent bryophytes, but one that also makes them particularly vulnerable to atmospheric pollution (Dollard et al., 1983; Unsworth, 1984; Fowler, Duyzer, and Baldocchi, 1991).

GROWTH-FORM, TRANSLOCATION, AND GROWTH

During (1990) and others have emphasized the importance of clonal growth in bryophytes. The internal conduction of water does not have the central role in bryophytes that it has for vascular plants, and lack of a root system in bryophytes removes a major demand for translocation of photosynthate and other materials. However, there are still the demands of new shoot growth, clearly illustrated by some of the larger acrocarps. In Polytrichaceae, rhizoid strands play a major part in establishment and vegetative spread (Wigglesworth, 1946), but innovations from established shoots are also important in perennation and vegetative reproduction (Longton and Greene, 1967; Longton, 1972; Collins, 1976; Hobbs and Pritchard, 1987). Clumps of *Dawsonia superba* are "maintained and extended by vegetative reproduction of new shoots from the rhizomes" (Green and Clayton-Greene, 1981), but here, as in *Polytrichum*, new shoots are probably formed mainly from the branched rhizoid strands rather than by branching of true "rhizomes" (Meusel, 1935). In the Mniaceae and in *Rhodobryum roseum*, basal innovations begin growth as plagiotropic rhizome-like shoots, turning upwards to form a new orthotropic leafy shoot perhaps some centimeters away. There are numerous less-striking examples (Meusel, 1935; Schofield and Hébant, 1984). The translocation of photosynthate from the older shoots to centers of new growth is readily demonstrated by radioactive labelling with ^{14}C (Collins and Oechel, 1974; Proctor, 1977). Except for Polytrichaceae with their specialized "leptoids" (Hébant, 1977), translocation of photosynthate and other metabolites must take place from cell to cell in relatively undifferentiated stem parenchyma. Amongst pleurocarpous mosses, similar "rhizomes" are particularly well developed in genera that have evolved dendroid branching systems, such as *Thamnobryum* and *Climacium* (Meusel, 1935). The shoot systems of these pleurocarps are sympodial although their capsules are lateral, and in fact sympodial branching is rather common in pleurocarpous mosses. *Hylocomium splendens* often shows a largely sympodial development of new shoots.

ADAPTIVE RADIATION AND EVOLUTIONARY CONVERGENCE, AND GENETIC AND PLASTIC VARIATION AT THE SPECIES LEVEL

Two very general principles of evolving life are apparent among bryophyte branching patterns: parallelism and adaptive radiation. Superficially very similar branching systems and life-form adaptations can be seen among leafy liverworts and mosses in spite of the morphological, anatomical, and physiological differences between these two groups, and their long evolutionary isolation. It is remarkable that the generally dorsiventral leafy liverworts have evolved tall turf (*Herberta*), short turf (*Marsupella*), small cushion (*Gymnomitrion*), and even pendant (tropical *Frullania* and *Lejeunea* species, *Bryopteris*) forms, as well as the mats and wefts that seem their principal métier (Mägdefrau, 1982).

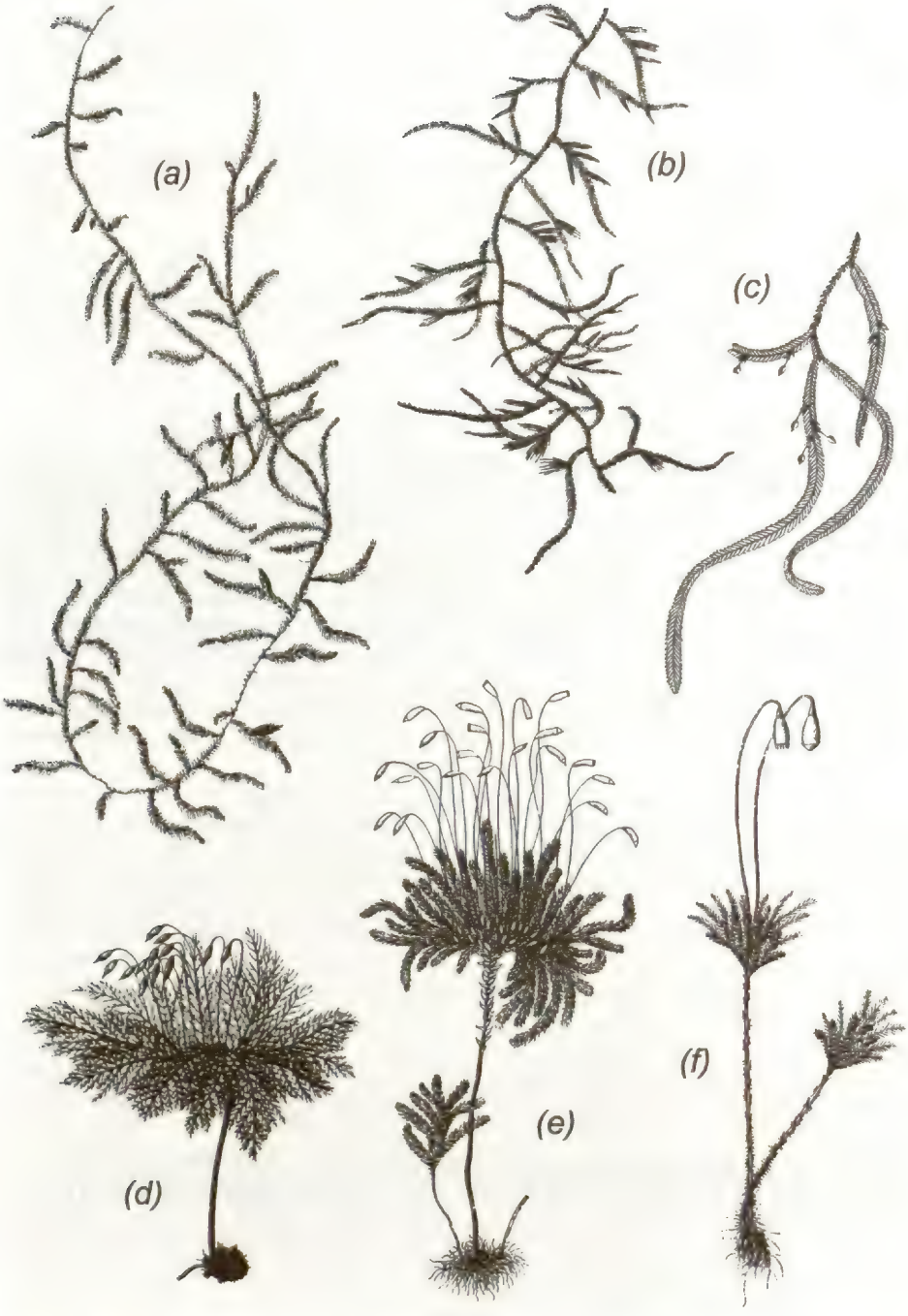


Figure 9. Adaptive convergence: pendant and dendroid life-forms. (a) *Pilotrichopsis dentata* (Cryphaeaceae) × 0.9; (b) *Papillaria helictophylla* (Meteoriaceae) × 0.9; (c) *Phyllogonium serra* (Phyllogoniaceae) × 0.9; (d) *Hypopterygium filiculiforme* (Hypopterygiaceae) × 0.9; (e) *Sciadocladus kerryi* (Hypnodendraceae) × 0.9; (f) *Leucolepis acanthoneura* (Mniaceae) × 1. From Brotherus (1909).

There is less overlap in life-forms between the acrocarpous and pleurocarpous mosses. Cushion and turf life-forms are found predominantly amongst the acrocarpous families, and mat and weft life-forms amongst the pleurocarps. In both cases, similar life-forms are found within diverse families, and most families show at least some diversity of life-form within limits set by their basic growth-pattern. However, amongst acrocarpous mosses some species of *Plagiomnium* (e.g., *P. cuspidatum*, *P. longirostrum*; Koponen, 1968) and species of *Racomitrium* (e.g., *R. lanuginosum, R. canescens*, and *R. fasciculare*) have life-form characteristics that bring them functionally close to the pleurocarps, and illustrate the kind of selection pressures involved in evolution of pleurocarpy (Meusel, 1935; Buck and Vitt, 1986; Mishler and De Luna, 1991). Several of the *Racomitrium* species are very plastic; thus *R. heterostichum* (sensu Frisvoll, 1988) forms tight small cushions on exposed mountain rocks, but grows in open rough mats on lightly shaded rocks amongst bracken or in open woodland. That pleurocarpy has evolved independently several times is shown by the occurrence of isolated pleurocarpous genera in a number of predominantly acrocarpous families (Vitt, 1984), and "pseudo-pleurocarps" still more or less clearly showing their acrocarp origins are found in the Grimmiaceae (*Racomitrium, Schistidium*), Pottiaceae (*Cincidotus*), Bryaceae (*Mielichhoferia*), Bartramiaceae (*Breutelia*), and other families. Conversely, *Orthotrichum* shows features that enabled Meusel (1935) to argue its pleurocarp origins. So-called cladocarpous mosses, with archegonia borne at the ends of leafy lateral branches, occur in both predominantly acrocarpous and predominantly pleurocarpous families. In *Fissidens*, basal innovations develop into either sterile or fertile shoots. The sterile shoots may be annual, or may go on to produce further growth-segments in successive years. In some species archegonial shoots are as long as the annual sterile shoots, bearing terminal capsules. In others, the archegonial shoots remain minute, so the capsules are borne apparently laterally on the parent shoot. Thus a range of states from "acrocarpy" to "pleurocarpy" can be seen in the one genus (Meusel, 1935). Meusel (1935) and Mishler and De Luna (1991) stress the difficulty of interpreting branching systems on the appearance of mature shoots alone; detailed developmental study may be needed before sound morphological and taxonomic conclusions can be drawn.

Amongst pleurocarpous mosses, dendroids such as *Climacium* and some erect-growing Amblystegiaceae have evolved growth-habit and branching patterns which, in life-form terms, bring them close to the tall-turf acrocarps. The dendroid habit has certainly arisen repeatedly, probably driven by selection pressure for overtopping competitors and leaf-litter, and preempting space (Figure 9). Dendroid growth-forms occur in a number of pleurocarpous families, and the big pleurocarpous dendroids find parallels in the acrocarpous *Dendroligotrichum* (Polytrichaceae) and *Leucolepis* (Mniaceae). Tuomikoski and Koponen (1979) remark on the diversity of branching patterns within the genera *Calliergon* and *Drepanocladus*, neither of which as conventionally delimited is now considered to be natural. Shoots of *Calliergon giganteum* are radially symmetrical, and those of *Drepanocladus procerus* hardly less so, whereas the branching of *Calliergonella cuspidata* and *Drepanocladus aduncus* is regularly complanate. It is easy to visualize selection favoring orthotropic growth and radial symmetry in wet sedge-fen habitats—situations in which tall-turf and large-cushion Bryales (e.g., *Bryum pseudotriquetrum, Rhizomnium* spp., *Plagiomnium* spp., *Aulacomnium palustre*) are often prominent, and favoring complanate branching in rather drier habitats with a lower and more open field-layer. Complanate versus radial branching can in fact provide a useful and constant character separating pairs of related genera or species-groups. Another example within the Amblystegiaceae is provided by the markedly radial *Cratoneuron filicinum* and the much more strictly complanate *Palustriella commutata* (Ochyra, 1989), which could be seen as reflecting the more eutrophic and competitive habitats of *C. filicinum* and the typically open, calcareous but nutrient-poor springs and flushes favored by *P. commutata*.

In general, plastic variation affects such characteristics as extension growth and density of branching, but not the fundamental architecture of the plant (Birse, 1957; Berthier, 1965, 1972; Glime and Raeymaekers, 1987; Bates, 1988). Its effect can nevertheless be very striking, and sometimes taxonomically confusing, as it is in the *Racomitrium heterostichum* group (Frisvoll, 1988). Similar phenotypic differences may be wholly or partly genetically determined in one case, and the consequence of plasticity in another. Thus the differences between the aquatic *Cryphaea lamyana* and *Isothecium holtii* and the terrestrial *C. heteromalla* and *I. myosuroides* largely parallel the plastic differences seen within *Cinclidotus fontinaloides* or *Schistidium alpicola* from the wet and dry extremes of their ecological ranges. Taxonomic problems that arise in this way can only be resolved by detailed critical field study, and experiment.

CONCLUSION

The fossil record suggests that the early evolution of land plants was a scene of rather rapid adaptive radiation, out of which evolved the diverse groups of bryophytes and pteridophytes that exist today. Many groups of early plants were faced by similar constraints and opportunities, and by the diverse selection pressures imposed by the physical environment and competing organisms. We see nowadays the end-products of diversification into different ecological niches and life-strategies, and also of the parallel evolution of different groups of early land-plants into comparable niches and strategies. In evolution as in engineering, the same problem can often be solved with equal effectiveness in different ways—and once a particular solution is established, the basic pattern cannot readily be changed. There are thus certain general morphological features of bryophytes, such as the absence of roots and the common tendency to develop complanate plagiotropic branching systems, which reflect common selection pressures inherent in their particular strategy of adaptation to land life. On the other hand, various features in which the major bryophyte groups differ, such as the detailed patterns of apical segmentation and branching, represent different but evolutionarily equally valid responses by different evolving groups to similar environmental challenges.

The simple contrast between the basic adaptive pattern of bryophytes and vascular plants implied in the introduction is of course an oversimplification. Filmy ferns such as *Hymenophyllum* simulate bryophytes in their form and eco-physiological adaptation, and there are desiccation-tolerant poikilohydric species amongst clubmosses, ferns, and flowering plants. Acrocarpous mosses such as *Dawsonia* and the large *Polytrichum* species, with their functionally rhizome-like underground rhizoid strands and well-developed internal conducting systems (Hébant, 1977), approach vascular plants in structure and function. In fact, true underground axes occur rather widely in bryophytes. The "rhizomes" of large mosses such as *Rhodobryum, Plagiomnium*, and *Climacium* are well known (Meusel, 1935). *Haplomitrium, Takakia*, and a few Metzgeriales (e.g., *Podomitrium, Hymenophyton*) and Jungermanniales (e.g., *Lembidium, Wettsteinia*) produce leafless underground axes that may function essentially as water-absorbing "roots" (Grubb, 1970; Schuster, 1984b). Amongst mosses, extensive underground axes occur, for instance, in the small acrocarp *Cheilothela chloropus* (Porley, 1992). Some liverworts produce flagelliform shoots that grow down into the substratum to form intricately interlacing systems of slender axes which at least in some species (e.g., *Kurzia pauciflora, Odontoschisma sphagni*) serve for perennation and survival (Duckett and Clymo, 1988). However, these various underground structures have evidently developed in response to different functions and selection pressures. There is no indication that they are homologous, or part of the sort of general trend towards bipolar organization seen amongst

vascular plants (Rothwell, 1994). Crandall-Stotler (1980) has suggested that mosses and primitive pteridophytes arose from a common vascularized progenitor, a view that accords with a number of features of mosses, including their long-lived bipolar sporophytes, and the widespread occurrence of differentiated central strands in the stems of their gametophytes (Hébant, 1977). If this is so, then the mosses are evolutionarily reduced in terms of their vascularization, but we concur with her view that in other respects the various bryophyte groups are, in their own adaptive context, highly evolved and advanced plants.

Much of the immediately obvious diversity in bryophyte branching patterns can readily be related to eco-physiological adaptation to the diverse environments in which bryophytes occur at the present day. Comparable selection pressures must have operated in the past, and such major growth-form contrasts and patterns as the acrocarp/pleurocarp distinction in mosses and the unique structure of *Sphagnum* probably first evolved as ecologically based life-form differences. They have become a genetically fixed part of the ontogeny of their respective groups, often to undergo extensive adaptive radiation and diversification in their turn. It is interesting that the adoption of a plagiotropic habit implied in pleurocarpy appears to be a relatively late but recurrent evolutionary development in mosses (Vitt, 1984), whereas it is a basic part of the architecture of the great majority of liverworts. Stabilizing selection will tend to integrate successful adaptations into the ontogenetic pattern of the organism in the course of time, so that they become to a greater or less degree developmental constraints on subsequent evolution (Smith et al., 1985). There is thus no absolute line between (innate) growth-form and (adaptive) life-form, although the distinction remains useful and important in considering the adaptive relationships and systematics of present-day bryophytes. Because bryophytes are close to the scale of the atmospheric laminar boundary-layer, their life-forms are physiologically an essential part of their structure. The colony, not the individual shoot, is usually the effective functional unit, and the unit upon which selection operates. Some indications have been given in this paper of the kind of quantitative relationships that may exist between bryophyte life-forms and factors of the physical environment. Much more measurement, theoretical analysis, and experimentation is needed to understand the adaptive relationships of bryophyte shoot systems to their environments, and the selection pressures that these imply.

The interplay of adaptive radiation and convergence in bryophyte evolution, and of genetic adaptation and phenotypic plasticity in bryophyte growth, have already been touched upon. All of these have their influence on bryophyte branching patterns, and on the systematic conclusions that may be drawn from them (Koponen, 1982). Perhaps hardly any features of bryophyte branching patterns are wholly free of ecological implications, but the fundamental differences in branching between the major bryophyte groups are in effect almost pure systematic information. Within the larger groups of bryophytes—the liverworts and the true mosses—the differences in branching patterns tend to become less "fundamental" and more obviously and immediately related to ecological adaptation as one descends the systematic hierarchy. At high levels of the hierarchy, the main difficulty may be to trace "fundamental" differences amongst the "noise" of subsequent adaptive radiation. Here, recent developments in biochemical and molecular techniques open the prospect of new insights and new evidence to test and extend the conclusions of traditional systematics. At the level of genus or species, the problem more often lies in distinguishing genetically determined and stable differences from those due to plasticity or susceptible to rapid and local selective change. This is traditional ground for biosystematics, but here too, modern methods have much to offer in analyzing the genetic basis and systematic value of the morphological variation of which branching patterns are a part.

LITERATURE CITED

Alpert, P. 1988. Survival of a desiccation-tolerant moss, *Grimmia laevigata*, beyond its observed microdistributional limit. J. Bryol. 15: 219–227.

Asakawa, Y. 1986. Chemical relationships between algae, bryophytes and pteridophytes. J. Bryol. 14: 59–70.

Basile, D. V. 1967. The influence of hydroxy-L-proline on ontogeny and morphogenesis of the liverwort, *Scapania nemorosa*. Amer. J. Bot. 54: 977–983.

_____. 1970. Hydroxy-L-proline and 2-2′dipyridyl-induced phenovariations in the liverwort *Nowellia curvifolia*. Science 170: 1218–1220.

_____. 1974. A possible role for hydroxyproline-proteins in leafy liverwort phylogeny. J. Hattori Bot. Lab. 38: 91–98.

_____. 1979. Hydroxyproline-induced changes in form, apical development, and cell-wall protein in the liverwort *Plagiochila arctica*. Amer. J. Bot. 66: 776–783.

_____. 1990. Morphoregulatory role of hydroxyproline-containing proteins in liverworts. Pp. 225–243 *in* R. N. Chopra and S. S. Bhatla (eds.), Bryophyte Development: Physiology and Biochemistry. CRC Press, Boca Raton, Florida.

_____ and M. R. Basile. 1990. Hydroxyproline metabolism and hydroxyproline-containing glycoproteins in leafy liverworts. Pp. 275–288 *in* H. D. Zinsmeister and R. Mues (eds.), Bryophytes: Their Chemistry and Taxonomy. Clarendon Press, Oxford.

Bates, J. W. 1987. Nutrient retention in *Pseudoscleropodium purum* and its relation to growth. J. Bryol. 14: 565–580.

_____. 1988. The effect of shoot spacing on the growth and branch development of the moss *Rhytidiadelphus triquetrus*. New Phytol. 109: 492–504.

_____. 1990. Interception of nutrients in wet deposition by *Pseudoscleropodium purum*: an experimental study of uptake and retention of potassium and phosphorus. Lindbergia 15: 93–98.

_____ and A. M. Farmer. 1990. An experimental study of calcium acquisition and its effects on the calcifuge moss *Pleurozium schreberi*. Ann. Bot. (London) 66: 87–96.

Berthier, J. 1965. Influence du milieu sur la ramification du *Fontinalis antipyretica* L. Compt. Rend. Hebd. Séances Acad. Sci. 260: 4046–4049.

_____. 1972. Recherches su la structure et le developpement de l'apex du gamétophyte feuillé des mousses. Rev. Bryol. Lichénol. 38: 421–451.

Birse, E. L. 1957. Ecological studies on growth-form in bryophytes. II. Experimental studies on growth-form in mosses. J. Ecol. 45: 721–733.

Bridel [-Brideri], S. E. 1826–1827. Bryologia Universa seu Systematica ad Novam Methodum Dispositio, Historia et Descriptio omnium Muscorum Frondosorum Hucusque cognitorum cum Synonymia ex Auctoribus Probatissimis. Volumes 1–2. J. A. Barth, Leipzig.

Brotherus, V. F. 1909. Musci. III. Unterklasse Bryales. II. Spezieller Teil. Pp. 277–1172 *in* A. Engler (ed.), Die Natürlichen Pflanzenfamilien I(3). W. Engelmann, Leipzig.

Brown, D. H. 1982. Mineral nutrition. Pp. 383–444 *in* A. J. E. Smith (ed.), Bryophyte Ecology. Chapman and Hall, London.

_____ and J. W. Bates. 1990. Bryophytes and nutrient cycling. Bot. J. Linn. Soc. 104: 129–147.

Buck, W. R. and D. H. Vitt. 1986. Suggestions for a new familial classification of pleurocarpous mosses. Taxon 35: 21–60.

Clymo, R. S. and P. M. Hayward. 1982. The ecology of *Sphagnum*. Pp. 30–78 *in* A. J. E. Smith (ed.), Bryophyte Ecology. Chapman and Hall, London.

Collins, N. J. 1976. Growth and population dynamics of the moss *Polytrichum alpestre* in the maritime Antarctic. Oikos 27: 389–401.

_____ and W. C. Oechel. 1974. The pattern of growth and translocation in a tundra moss *Polytrichum alpinum*. Canad. J. Bot. 52: 355–363.

Crandall, B. J. 1969. Morphology and development of branches in the leafy Hepaticae. Beih. Nova Hedwigia 30: 1–261.

Crandall-Stotler, B. 1972. Morphogenetic patterns of branch formation in the leafy Hepaticae—a resumé. Bryologist 75: 381–403.

_____. 1980. Morphogenetic designs and a theory of bryophyte origins and divergence. BioScience 30: 580–585.

_____. 1981. Morphology/anatomy of Hepatics and Anthocerotes. Advances Bryol. 1: 315–376.

_____. 1984. Musci, Hepatics, and Anthocerotes: an essay on analogues. Pp. 1093–1129 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

_____. 1986. Morphogenesis, developmental anatomy, and bryophyte phylogenetics: contraindications of monophyly. J. Bryol. 14: 1–23.

Crum, H. A. 1991. Liverworts and Hornworts of Southern Michigan. Univ. Michigan Press, Chicago.

Daniels, R. E. and A. Eddy. 1990. Handbook of European Sphagna. 2nd impression. Her Majesty's Stationery Office, London.

Dollard, G. J., M. H. Unsworth, and M. J. Harvey. 1983. Pollutant transfer in upland regions by occult precipitation. Nature 302: 241–243.

Duckett, J. G. 1986. Ultrastructure in bryophyte systematics and evolution. J. Bryol. 14: 25–42.

_____ and R. S. Clymo. 1988. Regeneration of bog liverworts. New Phytol. 110: 119–127.

During, H. J. 1979. Life strategies of bryophytes: a preliminary review. Lindbergia 5: 2–19.

_____. 1990. Clonal growth patterns among bryophytes. Pp. 153–176 *in* J. van Groenendael and H. de Kroon (eds.), Clonal Growth in Plants. SPB Academic Publishing, The Hague.

_____. 1992. Ecological classifications of bryophytes and lichens. Pp. 1–31 *in* J. W. Bates and A. M. Farmer (eds.), Bryophytes and Lichens in a Changing Environment. Oxford Univ. Press, Oxford.

Evans, A. W. 1912. Branching in the leafy Hepaticae. Ann. Bot. (London) 26: 1–37.

Fowler, D., J. H. Duyzer, and D. D. Baldocchi. 1991. Inputs of trace gases, particles, and cloud droplets to terrestrial surfaces. Proc. Roy. Soc. Edinburgh, B 97: 35–59.

Frey, W. 1970. Blattentwicklung bei Laubmoosen. Nova Hedwigia 20: 463–556.

_____. 1981. Morphologie und Anatomie der Laubmoose. Advances Bryol. 1: 399–477.

Frisvoll, A. A. 1988. A taxonomic revision of the *Racomitrium heterostichum* group (Bryophyta, Grimmiales) in N. and C. America, N. Africa, Europe, and Asia. Gunneria 59: 1–289.

Gams, H. 1959. Remarques sur les affinités entre les Mousses primitives. Rev. Bryol. Lichénol. 28: 326–329.

Gimingham, C. H. and E. M. Birse. 1957. Ecological studies on growth-form in bryophytes. I. Correlations between growth-form and habitat. J. Ecol. 45: 533–545.

_____ and E. T. Robertson. 1950. Preliminary investigations on the structure of bryophytic communities. Trans. Brit. Bryol. Soc. 1: 330–344.

Glime, J. M. and G. Raeymaekers. 1987. Temperature effects on branch and rhizoid production in six species of *Fontinalis*. J. Bryol. 14: 779–790.

Green, T. G. A. and K. A. Clayton-Greene. 1981. Studies on *Dawsonia superba* Grev. II. Growth rate. J. Bryol. 11: 723–731.

Grime, J. P., E. R. Rincon, and B. E. Wickerson. 1990. Bryophytes and plant strategy theory. Bot. J. Linn. Soc. 104: 175–186.

Grubb, P. J. 1970. Observations on the structure and biology of *Haplomitrium* and *Takakia*, bryophytes with roots. New Phytol. 69: 303–326.

Hagerup, O. 1935. Zur Periodizität im Laubwechsel der Moose. Kongel. Danske Vidensk. Selsk. Biol. Meddels. 11: 3–87.

Hébant, C. 1977. The Conducting Tissues of Bryophytes. J. Cramer, Vaduz.

Herzog, T. 1926. Geographie der Moose. G. Fischer, Jena.

Hill, M. O. 1978. 1. Sphagnopsida. Pp. 30–78 *in* A. J. E. Smith, The Moss Flora of Britain and Ireland. Cambridge Univ. Press, Cambridge, U.K.

Hobbs, V. J. and N. M. Pritchard. 1987. Population dynamics of the moss *Polytrichum piliferum* in North-east Scotland. J. Ecol. 75: 177–192.

Hoffman, G. R. 1972. The accumulation of cesium-137 by cryptogams in a *Liriodendron tulipifera* forest. Bot. Gaz. 133: 107–119.

Horikawa, Y. and H. Ando. 1952. A short study on the growth-forms of bryophytes and its ecological significance. Hikobia 1: 119–129.

Horn, H. S. 1971. The Adaptive Geometry of Trees. Princeton Univ. Press, Princeton, NJ.

Ingram, H. A. P. 1984. Hydrology. Pp. 67–158 *in* A. J. P. Gore (ed.), Ecosystems of the World: 4A. Mires: Swamp, Bog, Fen and Moor. General Studies. Elsevier, Amsterdam.

Jendralski, U. 1955. Die Jahresperiodizität in der Entwicklung der Laubmoose im Rheinlande. Decheniana 108: 105–163.

Koponen, T. 1968. Generic revision of Mniaceae Mitt. (Bryophyta). Ann. Bot. Fenn. 5: 117–151.

_____. 1982. Rhizoid topography and branching patterns in moss taxonomy. Pp. 95–99 *in* P. Geissler and S. W. Greene (eds.), Bryophyte Taxonomy. Beih. Nova Hedwigia 71.

Krassilov, V. A. and R. M. Schuster. 1984. Palaeozoic and Mesozoic fossils. Pp. 1172–1191 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

Lackner, L. 1939. Über die Jahresperiodizität in der Entwicklung der Laubmoose. Planta 29: 534–616.

Lewis, K. and A. J. E. Smith. 1977. Studies on some bulbiliferous species of *Pohlia* section *Pohliella*. I Experimental investigations. J. Bryol. 9: 539–556.

_____ and _____. 1978. Studies on some bulbiliferous species of *Pohlia* section *Pohliella*. II. Taxonomy. J. Bryol. 10: 9–27.

Limpricht, K. V. 1890–1904. Die Laubmoose. Volume. 4 *in* L. Rabenhorst (ed.), Kryptogamenflora Deutschlands, Oesterreichs, und der Schweiz. E. Kummer, Leipzig.

Longton, R. E. 1972. Growth and reproduction in Northern and Southern Hemisphere populations of the peat-forming moss *Polytrichum alpestre* with reference to the estimation of productivity. Proc. 4th Internat. Peat Congress, I–IV: 259–275. Helsinki.

_____ and S. W. Greene. 1967. The growth and reproduction of *Polytrichum alpestre* Hoppe on South Georgia. Philos. Trans., Ser. B 252: 295–322.

_____ and R. M. Schuster. 1984. Reproductive biology. Pp. 386–462 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 1. Hattori Botanical Laboratory, Nichinan.

Mägdefrau, K. 1969. Die Lebensformen der Laubmoose. Vegetatio 16: 295–297.

_____. 1982. Life-forms of bryophytes. Pp. 45–58 *in* A. J. E. Smith (ed.), Bryophyte Ecology. Chapman and Hall, London.

Meusel, H. 1935. Wuchsformen und Wuchstypen der europäischen Laubmoose. Nova Acta Leop. 3: 123–277.

Mishler, B. D. and E. De Luna. 1991. The use of ontogenetic data in phylogenetic analyses of mosses. Advances Bryol. 4: 121–167.

Monteith, J. L. and M. H. Unsworth. 1990. Principles of Environmental Physics. 2nd edition. Edward Arnold, London.

Monsi, M., Z. Uchijima, and T. Oikawa. 1973. Structure of foliage canopies and photosynthesis. Annual Rev. Ecol. Syst. 4: 301–327.

Ochyra, R. 1989. Animadversions on the moss genus *Cratoneuron* (Sull.) Spruce. J. Hattori Bot. Lab. 67: 203–242.

Parihar, N. S. 1965. An Introduction to Embryophyta. I. Bryophyta. 5th edition. Central Book Depot, Allahabad.

Porley, R. D. 1992. *Cheilothela chloropus* (Brid.) Lindb. with underground axes. J. Bryol. 17: 164–165.

Proctor, M. C. F. 1977. Evidence on the carbon nutrition of moss sporophytes from $^{14}CO_2$ uptake and the subsequent movement of labelled assimilate. J. Bryol. 9: 375–386.

_____. 1981. Diffusion resistances in bryophytes. Pp. 219–229 *in* J. Grace, E. D. Ford, and P. G. Jarvis (eds.), Plants and Their Atmospheric Environment. Blackwell Scientific Publications, Oxford.

_____. 1982. Physiological ecology: water relations, light and temperature responses, carbon balance. Pp. 333–381 *in* A. J. E. Smith (ed.), Bryophyte Ecology. Chapman and Hall, London.

_____. 1984. Structure and ecological adaptation. Pp. 9–37 *in* A. F. Dyer and J. G. Duckett (eds.), The Experimental Biology of Bryophytes. Academic Press, London.

_____. 1990. The physiology of bryophyte production. Bot. J. Linn. Soc. 104: 61–77.

Raven, J. A. 1977. The evolution of vascular land plants in relation to supracellular transport processes. Advances Bot. Res. 5: 153–219.

_____. 1984. Physiological correlates of the morphology of early vascular plants. Bot. J. Linn. Soc. 88: 105–126.

Renzaglia, K. S. 1978. A comparative morphology and developmental anatomy of the Anthocerotophyta. J. Hattori Bot. Lab. 44: 31–90.

_____. 1982. A comparative developmental investigation of the gametophyte generation in the Metzgeriales (Hepatophyta). Bryophyt. Biblioth. 24: 1–253.

Richards, P. W. 1984. The ecology of tropical forest bryophytes. Pp. 1233–1270 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

Rieley, J. O., P. W. Richards, and A. L. D. Bebbington. 1979. The ecological role of bryophytes in a North Wales woodland. J. Ecol. 67: 497–527.

Rincon, E. 1988. The effect of herbaceous litter on bryophyte growth. J. Bryol. 15: 209–217.

Rothwell, G. W. 1994. Fossil history of branching patterns in vascular plants. Pp. 71–86 *in* P. C. Hoch and A. G. Stephenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard.

Schofield, W. B. 1985. Introduction to Bryology. Macmillan, New York.

_____ and C. Hébant. 1984. The morphology and anatomy of the moss gametophore. Pp. 627–657 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

Schuster, R. M. 1966. Hepaticae and Anthocerotae of North America, Volume 1. Columbia Univ. Press, New York.

_____. 1984a. Comparative anatomy and morphology of the Hepaticae. Pp. 762–891 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

_____. 1984b. Evolution, phylogeny and classification of the Hepaticae. Pp. 892–1070 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

_____. 1984c. Morphology, phylogeny and classification of the Anthocerotae. Pp. 1071–1092 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

_____. 1992. The Anthocertoae and Hepaticae of North America, Volume VI. Field Museum of Natural History, Chicago.

Smith, A. J. E. 1978. The Moss Flora of Britain and Ireland. Cambridge Univ. Press, Cambridge, U.K.

_____. 1990. The Liverworts of Britain and Ireland. Cambridge Univ. Press, Cambridge, U.K.

_____ and H. L. K. Whitehouse. 1978. An account of the British species of the *Bryum bicolor* complex, including *B. dunense* sp. nov. J. Bryol. 10: 29–47.

Smith, J. M., R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert. 1985. Developmental constraints and evolution. Quart. Rev. Biol. 60: 265–287.

Stebbins, G. L. and D. V. Basile. 1986. Phyletic phenocopies: a useful technique for probing the genetic and developmental basis of evolutionary change. Evolution 40: 422–425.

_____ and G. J. C. Hill. 1980. Did multicellular plants invade the land? Amer. Naturalist 115: 342–353.

Streeter, D. T. 1965. Seasonal variations in the nutrient content of carpets of *Acrocladium cuspidatum* (Hedw.) Lindb. Trans. Brit. Bryol. Soc. 4: 818–827.

Tamm, C. O. 1953. Growth, yield and nutrition in carpets of a forest moss (*Hylocomium splendens*). Meddeland. Statens Skogs-forskningsinst. 43: 1–140.

Taylor, T. N. 1982. The origin of land plants: a palaeobotanical perspective. Taxon 31: 155–177.

Tuomikoski, R. and T. Koponen. 1979. On the generic taxonomy of *Calliergon* and *Drepanocladus* (Musci, Amblystegiaceae). Ann. Bot. Fenn. 16: 213–227.

Unsworth, M. H. 1984. Evaporation from forests in cloud enhances the effects of acid deposition. Nature 312: 262–264.

Vitt, D. H. 1984. Classification of the Bryopsida. Pp. 696–757 *in* R. M. Schuster (ed.), New Manual of Bryology, Volume 2. Hattori Botanical Laboratory, Nichinan.

Walther, K. 1983. Syllabus der Pflanzenfamilien. V 2. Bryophytina. Laubmoose. Gebrüder Borntraeger, Berlin and Stuttgart.

Wigglesworth, G. 1946. Reproduction in *Polytrichum commune* L. and the significance of the rhizoid system. Trans. Brit. Bryol. Soc. 1: 4–13.

Williamson, M. H. and J. H. Lawton. 1991. Fractal geometry of ecological habitats. Pp. 69–86 *in* S. S. Bell, E. D. McCoy, and H. R. Mushinsky (eds.), Habitat Structure: The Physical Arrangement of Objects in Space. Chapman and Hall, London.

DEVELOPMENTAL THEMES IN VASCULAR PLANTS: FUNCTIONAL AND EVOLUTIONARY SIGNIFICANCE[1]

Donald R. Kaplan[2,3] and Paul A. Groff[2]

ABSTRACT. Two essential facets of the development and evolution of plant form are: *Organization* and *Construction*. Organization refers to the basic developmental theme or ground plan (Bauplan) of a group and reflects its evolutionary history. Construction refers to the functional or adaptational characteristics of the morphology of the plants. Ferns and flowering plants are examples of vascular plant groups that have contrasting Bauplans but converge in function. In ferns, the leaf is the dominant morphological and functional element of the shoot; in angiosperms, the shoot as a whole is specialized for the same functional roles. Angiospermic shoot analogs in ferns are possible because fern fronds have the capacity for (1) prolonged acroplastic growth and (2) forming shoot buds at an infinite number of leaf loci. In this article we highlight the concepts of organization and construction by describing and illustrating angiospermic shoot analogies in ferns for each of the following life forms: stoloniferous growth, the vining habit, the epiphytic habit, the floating aquatic habit, and reproductive organs. From these studies we conclude that, even though the Bauplans of these respective groups are distinctive and manifest from the earliest phases of their ontogeny, each ground plan has sufficient flexibility to allow specific adaptations to develop. Moreover, because we know so little of the basic mechanics of plant development, our emphasis on developmental themes is a more profitable perspective than those that emphasize developmental constraints.

INTRODUCTION

"With fronds like these, who needs anemones?" Anonymous.

Any attempt to evaluate the structural evolution of organisms must do so from two complementary perspectives: the organizational theme or Bauplan characteristic for the group and the functional or adaptive features of its structure. European morphologists have termed these two aspects *organization* and *construction,* respectively (Weber, 1958; Seilacher, 1970; Froebe and Classen, 1986). Organization refers to the morphological relationships of structure, reflecting the evolutionary history of the group, whereas construction refers to the functional role of these structural features. Thus organization and construction denote the homologous and analogous facets of organismal structure. They represent such complemen-

[1]We express our sincere appreciation to Dr. Linda Vorobik, who made the excellent drawings and labeled all illustrations, and to Jim Hendel, who made the majority of the fine photographs. We also express our appreciation to Dr. Richard Mueller, who supplied photographs from his work on *Lygodium,* and to Professor Dr. Wolfgang Hagemann for permission to use drawings of fern embryogenesis and leaf development from his previous publications and who provided copies of the unpublished Staatsexamen theses from his students in Heidelberg. Dr. Dawn Frame kindly supplied an excellent photograph of *Drynaria rigidula* in its native habitat in New Caledonia. We also thank Dr. Judith Croxdale, who critiqued an early draft of this article, and Ms. Eleanor Crump for her excellent editing of the manuscript. Special thanks go to Dr. Alan Smith, pteridologist in the University of California Herbarium, for his careful and critical reading of this manuscript and updating of fern nomenclature. Finally, we express our thanks to the staffs of the University of California Herbarium and Botanical Garden for the loan of specimens for photography and drawings illustrating this article.

[2]Departments of Plant Biology and Integrative Biology, University of California, Berkeley, Berkeley, California 94720, U.S.A.

[3]Author to whom correspondence should be directed.

tary evolutionary attributes that it is impossible to consider one without the other. Furthermore, in higher plants, as in animals, one finds marked convergences in construction between groups with vastly different modes of organization. The goal of this article is to illustrate these concepts by examining functional convergences between two different vascular plant groups with markedly contrasting ground plans, the ferns and the flowering plants (Figure 1), and to emphasize that any study of plant structural evolution must take into consideration both the organizational and constructional features of the groups concerned.

In this paper we are concerned principally with convergence in growth habit or life form between plant groups with characteristic morphologies. Thus we also underscore the principle that in the same way that the anatomical, i.e., cell-to-tissue, levels of organization are not predictive of morphological diversity (Kaplan and Hagemann, 1991), morphology, in turn, does not predict life-form diversity.

THE CONTRASTING BAUPLANS OF FERNS AND ANGIOSPERMS

Flowering plants and ferns represent the most structurally diverse members of their respective evolutionary alliances, seed plants and pteridophytes. Angiosperms are estimated to consist of some 241,000 species (Mabberley, 1987). They occupy virtually every habitat with a bewildering array of life forms, including trees, vines, aquatics, epiphytes, and parasites. With 8,450 species (Mabberley, 1987), the ferns (Filicopsida) are a considerably smaller group, but they are the one contemporary pteridophyte group that approaches the flowering plants in variety of life forms and habital diversity. The only life form not exhibited by ferns is the parasitic habit. As we illustrate below, this convergence in function between ferns and flowering plants occurs against the background of fundamental differences in ground plan.

The bodies of ferns and flowering plants are differentiated into the same major organ components—leaf, stem, and root—but differ significantly in the topography and elaboration of these organs. In a typical dicotyledonous model, shoot and root systems are spatially separated and diametrically opposed to one another with shoot branches axillary in insertion (Figure 2A). By contrast, the ferns do not develop a spatially separated root system but instead initiate roots exclusively from the shoot axis (Figure 2B). Goebel (1928–1933) coined the terms allorrhizic (meaning different rooting) and homorrhizic (meaning same rooting) to express this contrasting shoot-root relationship between seed plants (allorrhizic) and pteridophytes (homorrhizic). Moreover, in ferns the leaf is usually the largest and most conspicuous organ and plays the most significant biological role of the sporophyte phase (Figures 1, 2B). Ferns also differ in having predominantly extra-axillary branching. In the analogies between ferns and flowering plants the frond of ferns converges functionally with the shoot of angiosperms (Figure 1).

A clear example of the ground plan of ferns is illustrated by *Stenochlaena palustris* (Blechnaceae; Figure 3). Its shoot is differentiated into a dorsiventral, plagiotropic stem (rhizome) bearing pinnate fronds in a distichous phyllotaxis on its dorsal side and roots on its ventral side (Figure 3). While the leaf component is elaborated, each leaf has its own characteristic morphology (Figure 3). Even though the roots emerge some distance from the meristem, they too have their inception just below the shoot apex. Hence like leaf and stem, they originate in the zone of primary morphogenesis in the embryonic part of the shoot (Hagemann, 1967).

This fundamental Bauplan in ferns is manifest from their earliest phases of embryogenesis. Figure 4 illustrates stages of embryo development in the ferns *Hypolepis repens* (Figure 4A–E) and *Pentagramma triangularis* (Figure 4F and G). In contrast to vascular plant embryos with an endoscopic polarity, those of ferns do not exhibit a filamentous growth but undergo parenchyma formation directly (Figure 4A and B). Furthermore, the first organ

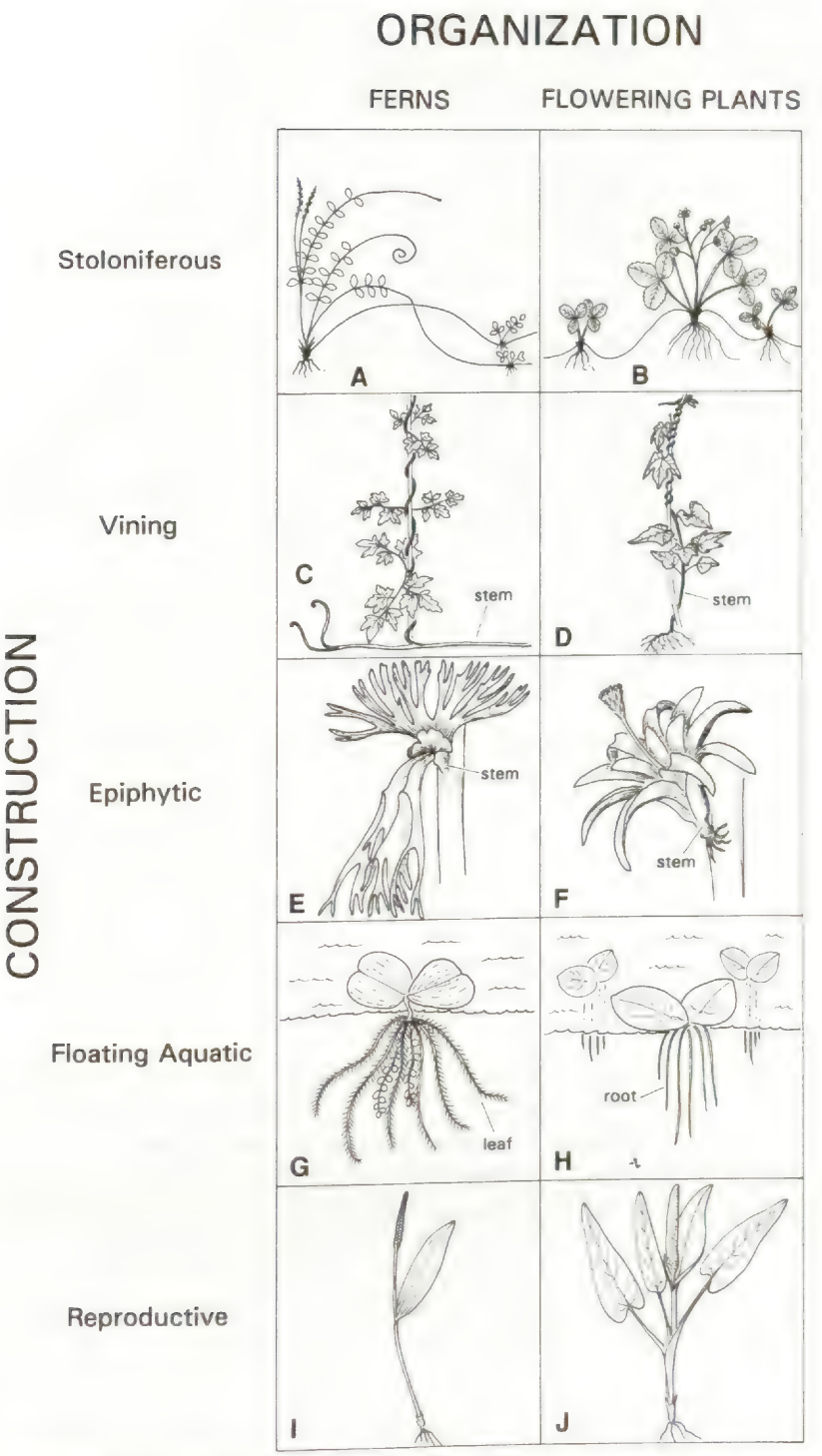


Figure 1. Illustration of the convergence in life forms (CONSTRUCTION) of ferns with flowering plants that have fundamentally different Bauplans (ORGANIZATION).

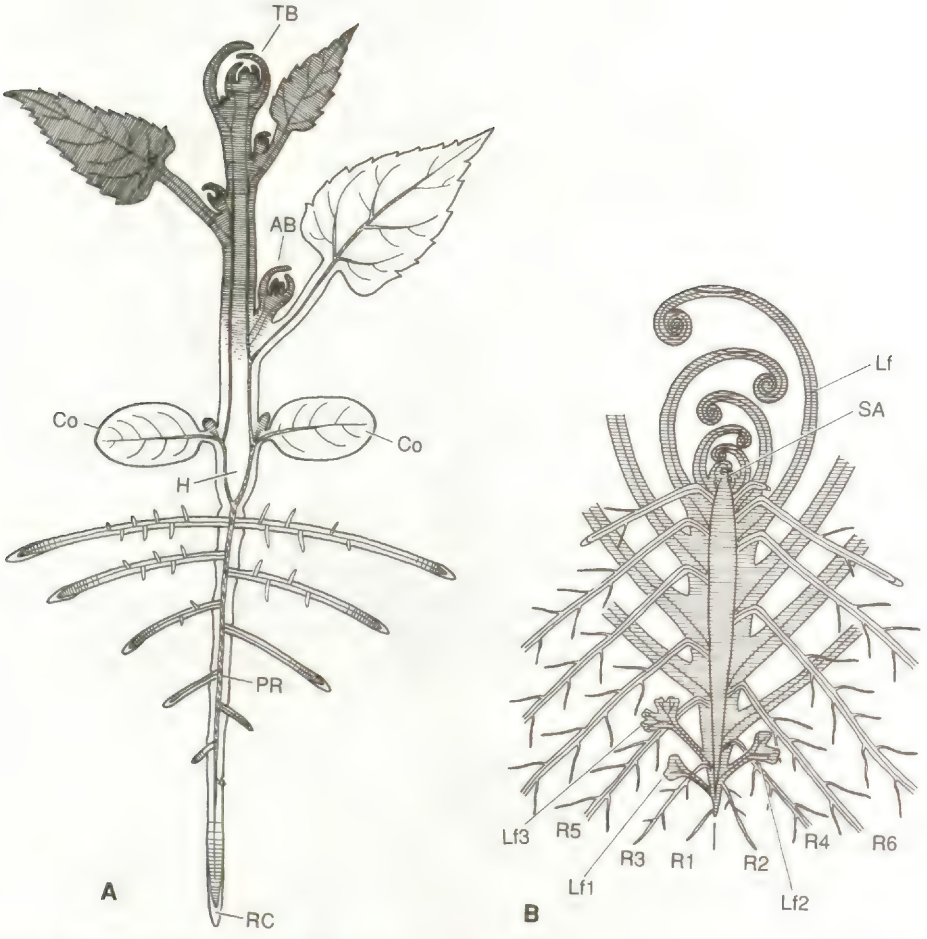


Figure 2. Median longitudinal sections of the bodies of a dicotyledonous flowering plant (A) and a fern (B), illustrating the developmental theme or Bauplan which is distinctive for each group. In the case of the dicotyledon (A), the spatially separated shoot and root systems (allorrhizy) express the basic bipolar nature, and shoot branching is axillary. By contrast, the fern is unipolar and its roots are initiated from the shoot in the vicinity of the shoot apical meristem (homorrhizy). AB, axillary bud; Co, cotyledon; H, hypocotyl; Lf1–3 fern juvenile leaves; Lf, adult frond; PR, primary root; R1–R6, fern roots numbered in the sequence of decreasing age. RC, root cap; SA, shoot apex; TB, terminal bud. [A: redrawn with permission from Troll (1937); B: redrawn with permission from Troll (1959).]

products are expressed before there is any significant axial elongation (Figure 4B). Reading from the most advanced stages back to their earliest differentiation (Figure 4G to 4B), the first leaf and root primordia are first evidenced by their diagnostic apical cell geometry and lineage patterns (Figure 4B–E). Since the leaf and root primordia are superposed, laterally emergent organs derived from the embryonic parenchymatic mass, the primordial embryonic body can be viewed as the shoot meristem, which will continue this iterative process of leaf and root inception during the entire life of the fern sporophyte (Figure 4B–G).

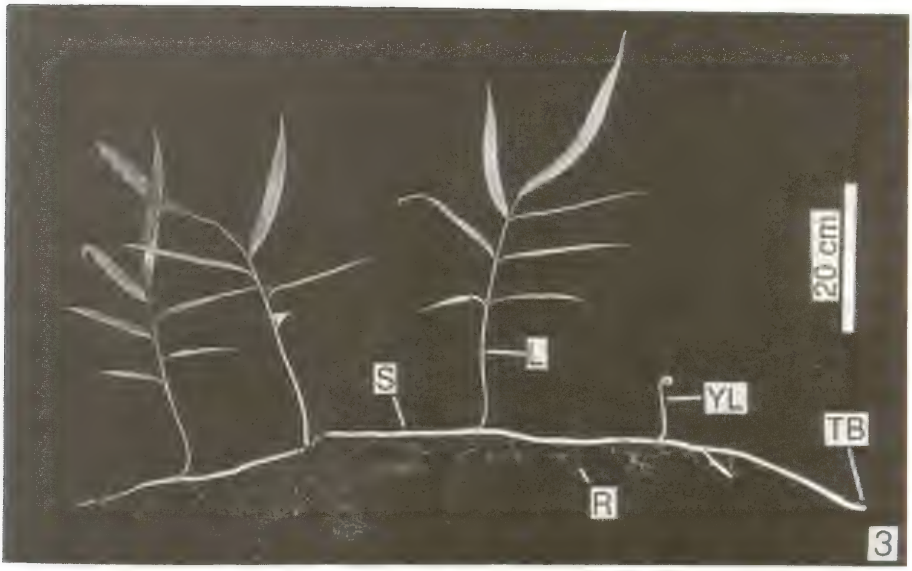


Figure 3. Fern *Stenochlaena palustris* (Blechnaceae), which exemplifies the organizational theme or ground plan for ferns. The plant body is differentiated organographically into leaf (L), stem (S), and roots (R). This fern has a plagiotropic axis that grows horizontally over the substrate, producing leaves in a distichous phyllotaxis on its dorsal surface and roots from its ventral surface. In this specimen, young, circinate leaves are evident (YL), as are a branch and the terminal bud (TB).

The shoot apical meristem is the distal pole of the embryo. Prior to the initiation of the next leaf the shoot apex enlarges to its plastochronic maximum with a divergence of about 180 degrees (Figure 4G). Thus leaf inception in ferns is so precocious that it occurs before the characteristic apical cell of the shoot apex is differentiated (Figure 4B). Nevertheless, the distal point of origin and predominantly erect growth of the young fern leaf primordium means that the young shoot apex initially is canted like that of other distichous shoots (Figure 4G; Kaplan, 1973).

As in the embryos of seed plants, the first root primordium in ferns is defined not only by the early differentiation of its tetrahedral apical cell but also by the periclinal wall insertions demarcating the first root cap cells (Figure 4E–G). The most marked contrast with seed plant embryos is that the root pole of ferns is not diametrically opposed to the shoot pole but is inserted at variable angles with the embryonic axis (Figure 4G). It is this unipolar character of the fern embryo that is the earliest expression of its homorrhizy. Hence even the first root primordium in a fern is interpreted to be a shoot-borne root and the first expression of the shoot origin of its subsequent roots. Because of their inherent unipolarity and the lack of formation of a spatially separated root axis, such pteridophytes are not considered to develop a root system comparable to their shoot system at the opposite pole. Instead they form roots as individual organs (which may branch) from the shoot axis or stem (Figure 2B). That this is not an abnormal or adventive condition is reinforced by its early expression during embryogenesis, which then sets the pattern for the remainder of the plant's development (Groff and Kaplan, 1988).

DEGREES OF ORGAN DIFFERENTIATION IN THE FERNS

When we state that the fronds of ferns exhibit functional analogies with the shoots of angiosperms, we assume that fern fronds and leaves in flowering plants are homologous

Figure 4. Median longitudinal sections of the stages of fern embryogenesis for *Hypolepis repens* (A–E) and *Pentagramma triangularis* (F–G), illustrating the origin of the basic Bauplan of the fern sporophyte during embryo development. AN, archegonial neck; Em, embryo; Lf, first leaf; R, first root; SA, shoot apical meristem. [Redrawn with permission from Hagemann (1984).]

organs, i.e., they are both phyllomes. However, some morphologists consider the acroplastic differentiation, prolonged apical growth, and highly branched blade surfaces exhibited by fern fronds as indications of their branch homologies and phylogenetic derivation from ancient branch or telome systems (Zimmermann, 1930; Bower, 1935; Eames, 1936; Bierhorst, 1968, 1969, 1971, 1974). If fern fronds really are branch rather than leaf homologs, then their functional correspondence with angiospermic shoots would be an expression of their homology rather than analogy. Since this is an important issue for our perspective, we summarize here the evidence that fern leaves (fronds) are leaf homologs and that fern shoots exhibit the same degree of organization and differentiation as those of angiosperms.

Despite their large, elaborated nature, from inception fern leaves are developmentally distinct from shoot branch meristems. Not only do the primordia of buds differ topographically from those of leaves but the bud meristems are also the sites of leaf inception. Moreover, when the earliest phases of leaf and branch origin are examined comparatively, it is clear that these two organ types are qualitatively distinct from inception. Contrary to previous assertions (Wardlaw, 1949), there are no stages where leaf and bud primordia are identical. For example, Figure 5 compares the stages of leaf and bud primordia for the climbing fern *Microgramma vacciniifolia* (Polypodiaceae) (Hirsch and Kaplan, 1974). Whereas the leaf primordium is distinguished by its precocious protuberance from periphery of the shoot apex and early definition of its lenticular apical cell (Figure 5C–G), branch primordia are barely evident at the earliest phases of development (Figure 5I–M). During its early development, the branch apex is sunken without a clear definition of its apical cell until the meristem becomes protuberant with the onset of extension (Figure 5J–N). Thus not only do fern branches occupy different positions than leaves and roots in fern shoots (i.e., ferns exhibit a phyllotaxy, a cladotaxy and a rhizotaxy), but they also express this determination from initiation (see review in Kaplan, 1977).

A leaf homology of fern fronds is also indicated by both the transectional symmetry of their primordia and the corresponding distribution of their meristems. Hagemann (1964, 1967, 1970, 1973) has indicated that in the vast majority of vascular plants, leaf primordia arise as dorsiventral, eumeristematic fractions of the shoot apex (Figure 6B). As these leaf primordia grow in length and breadth their dorsiventrality is manifest by their hyponastic curvature over the shoot apex (Figure 6A). Such hyponasty is an expression of the differential rate of elongation between the abaxial and adaxial sides of the leaf primordium. Rapid elongation on the abaxial side in continuity with the more rapidly differentiating cortex in the stem contrasts with the slower rate of cell enlargement and vacuolation on the adaxial side adjacent to the shoot apex (Figure 6A). Once tissue maturation reaches the leaf apex, elongation occurs faster on the adaxial side, resulting in erect or horizontal growth dependent upon the leaf's vernation.

In leaves with more limited apical meristematic activity, such as those in most seed plants, the initial abaxial vacuolation wave proceeds acropetally to the apex. Once the tip matures, differentiation proceeds basipetally with the final phases of tissue maturation. In the ferns, however, leaf maturation is strictly acropetal, and the apex retains its embryonic character for the duration of leaf development. The persistence of apical meristematic activity in fern leaves is marked by the retention of the hyponastic curvature of the leaf apex in the form of the crozier at its tip (Figure 6A). Such an adaxial coil indicates the embryonic status of the leaf's apex and also serves to protect the most embryonic and hence most desiccation-prone parts of the leaf. Once maturation spreads to the tip of the fern leaf, the growth rate on the adaxial surface catches up with that on the dorsal side and the crozier unrolls.

These initial waves of leaf maturation not only proceed from the abaxial to the adaxial side of the primordium, but also from its center to its periphery. As a result, the leaf's

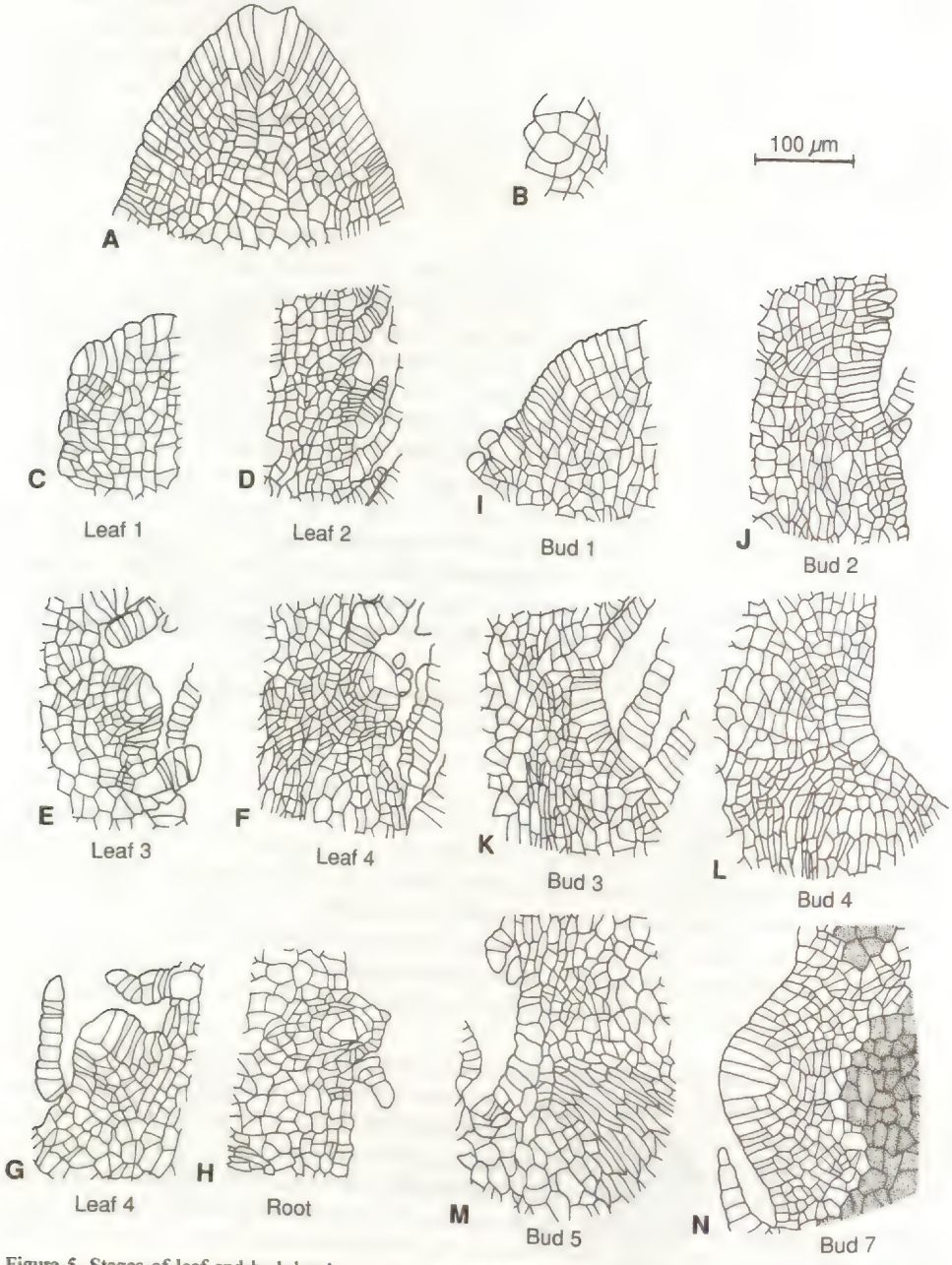


Figure 5. Stages of leaf and bud development in the fern *Microgramma vacciniifolia*, illustrating the differences between leaves and branches during their initiation and early development.—A. Median longisection of the shoot apex.—B. Transection of the shoot apex showing apical cell configuration.—C–G. Median radial longisections of stages of early leaf development in successively older plastochrons.—H. Median longisection of recently initiated root.—I–N. Median radial longisections of stages of bud initiation and development at successive plastochrons. Stippled cells in N bear tannins. [Reproduced with permission of the journal, from Hirsch and Kaplan (1974).]

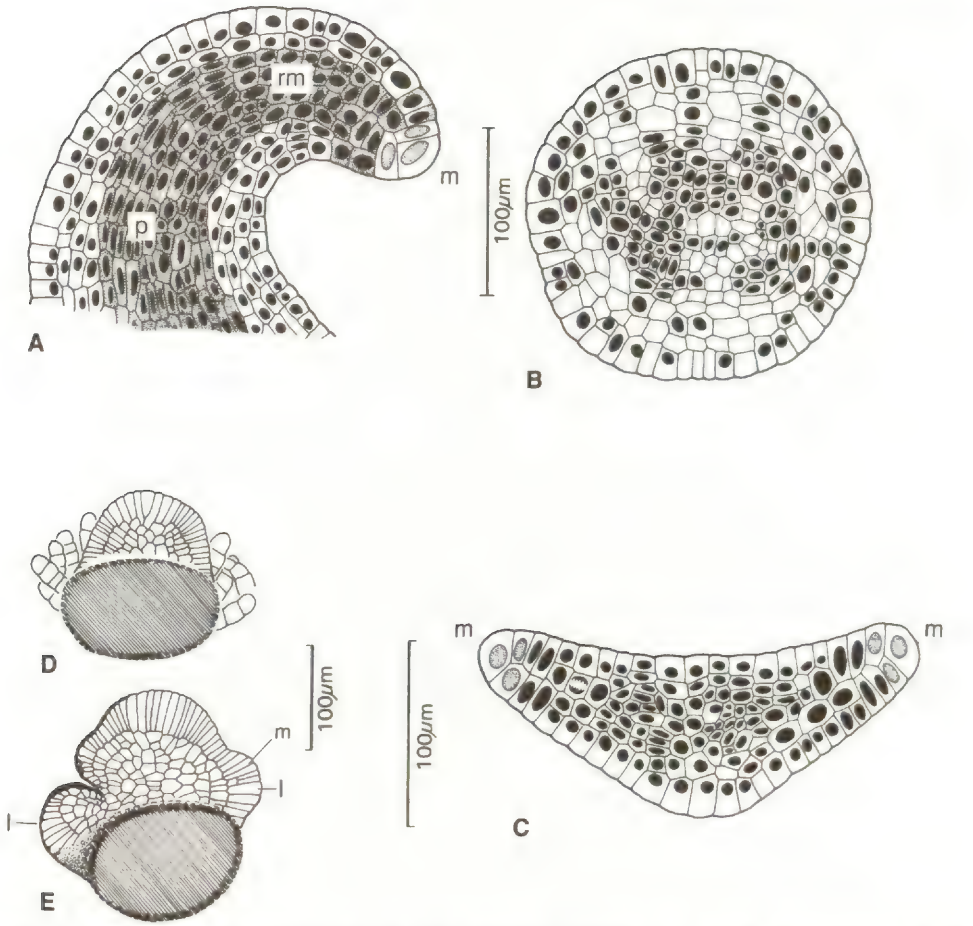


Figure 6. Features of leaf morphogenesis in the fern *Adiantum raddianum*.—A. Median longisection of the leaf apex showing the hyponastic curvature of the primordium and its expression of dorsiventrality as a result of differential growth on the abaxial versus adaxial leaf surfaces.—B. Transection of a young rachis unit.—C. Transection of pinna with its marginal meristems.—D–E. Surface view of leaf primordia with pinnae originating through fractionation of the marginal meristem. l, leaflet primordium; m, marginal meristem; p, procambium; rm, residual meristem. [Reproduced with permission from Hagemann (1992).]

meristem becomes confined as a eumeristematic band running over its apex and down the primordial flanks as a line separating its adaxial from abaxial surfaces (Figure 6B–C; Hagemann, 1964, 1970; Hagemann and Kaplan, 1989). The most conspicuous expression of the activity of this marginal meristem is its initiation of lobes or leaflets as fractions of that eumeristem (Figure 6D–E; Hagemann and Kaplan, 1989).

Since the leaf's marginal meristem occurs as a peripheral zone, when a lateral leaflet arises, the marginal meristem is carried equally around the leaflet periphery (Figure 6E). Because of this meristem symmetry, leaflets do not show the differential growth and hyponastic arching over the leaf apex in the way that leaves arch over their parent shoot apices (compare Figure 6E with 6A). Leaflets will show a curvature, of course, but this arching is in the dorsiventral plane of the leaf as a whole, i.e., in the transverse plane of the shoot. Thus the fronds

of ferns exhibit the same developmental symmetry relationships to their shoot axes as the leaves of seed plants, and these properties are the most fundamental indicator of their leaf rather than branch homology.

Additional evidence of the homology of fern leaves with those in angiosperms is the occurrence of the same range in leaf morphology as found in angiosperms. For example, in addition to pinnate fronds which superficially resemble seed-plant shoots, fern leaves range from determinate appendages which are simple in morphology (*Microgramma* and *Camptosorus*) to those that are pedately dissected (*Adiantum* and *Neocheiropteris*) to those with peltate blades (*Microgonium* and *Botrychium*). Moreover, even pinnate fronds exhibit a variation in the longitudinal symmetry expressed by regular variations in the degree of pinna outgrowth [i.e., acrotonic, mesotonic, and basitonic longitudinal symmetry (Troll, 1939)], which are independent of the direction of pinna inception. Pinna origin is strictly acropetal in fern fronds, but the degree of pinna extension does not necessarily reflect the sequence of pinna initiation. Instead it reflects differences in the overall shape of the blade. Thus the parallel in diversity of leaf forms between ferns and flowering plants further supports the homology of these appendages.

Finally, the fronds of ferns exhibit the same correlations between size and leaf complexity and axis primary thickening as all plant groups (Troll and Rauh, 1950; Hagemann, 1964). Because fern shoots do not have secondary thickening, they readily show the obconic form and ontogenetic changes in primary thickening of the shoot axis (Figure 2B). As an expression of this Erstarkungswachstum (Troll and Rauh, 1950), leaves initiated from the narrow stem regions are smaller and less elaborated than those from the thickened sectors (Crotty, 1955; Cutter, 1955). Furthermore, these integrated changes in leaf characters are correlated with the diameters of the shoot apical meristem and thus parallel the same type of heteroblastic development that has been documented for a range of angiosperms (Allsopp, 1965; Kaplan, 1973, 1980).

Thus, taken in aggregate, all these characteristics of fern leaves support their homology with angiosperm leaves and hence the concept that the fern fronds we describe here represent functional *convergences* with the shoots of seed plants.

CONVERGENCES IN CONSTRUCTION BETWEEN FERNS AND FLOWERING PLANTS

Stoloniferous Growth—The stoloniferous habit is a mechanism of lateral vegetative spread over a given surface. In flowering plants stolons or runners typically are aboveground lateral branches characterized by plagiotropic growth and elongated internodes (Figure 1B; Troll, 1937). They arise from the lower leaf axils of the parent shoot and, after varying degrees of plagiotropy, turn upright and undergo orthotropic growth as rosette shoots. Associated with this change in growth direction is a marked primary thickening and the initiation of shoot-borne roots, which anchor the new rosette (Figures 1B, 7; Troll, 1959). Since the thinner stolon axis can rot away, individual rosettes can become isolated as individual plants. Thus the stoloniferous life form is also a mechanism of clonal propagation (Figure 1B). *Saxifraga flagellaris* is a classical example of a stoloniferous life form in angiosperms (Figure 7).

Striking analogies to this stoloniferous habit are exhibited by numerous fern species. However, instead of the shoot being the runner, it is the elongated leaf which is stoloniferous (Figure 1A). In these plants the elongated blade or rachis arches over and bears a shoot bud at its apex (Figure 8). The bud then roots and establishes a new plantlet some distance from the parent rosette (Figure 8). However, instead of the apex of the stolon shoot in angiosperms turning upright and forming the daughter rosette, in the ferns a new shoot is initiated from the

leaf apex, which in turn becomes the new rosette. Also, like the shoot runner in flowering plants, death of the parent leaf connection results in the development of a new individual and hence a method of asexual propagation. Beck (1982) commented that in the case of *Anemia rotundifolia* such asexual reproduction can be considered a means of propagation when it is too dry for spore germination. Marchal (1965) also suggested that the stoloniferous habit in these ferns can be considered as the method of short-distance dispersal and spores the method of long-distance spread. Fern species that exhibit this stoloniferous growth mode and have been studied in some detail are *Camptosorus rhizophyllus* (Kupper, 1906; McVeigh, 1934; Yarbrough, 1936), *Anemia rotundifolia* (Kupper, 1906; Marchal, 1965; Beck, 1982), *Adiantum edgeworthii* (Kupper, 1906; Goebel, 1928–1933), *Asplenium barteri* (Marchal, 1965), *A. dregeanum* (Marchal, 1965), *A. emarginatum* (Marchal, 1965), *A. lauterbachii* (Kupper, 1906; Goebel 1928–1933), *A. oceanicum* (Kupper; 1906; Goebel, 1928–1933), *A. prolongatum* (Kupper, 1906; Marchal, 1965), *A. sandersonii* (Rösch, 1982), *Bolbitis heteroclita* (Marchal, 1965), *B. salicina* (Marchal, 1965), *Fadyenia fadyenii* (Marchal, 1965), *F. prolifera* (Kupper, 1906), and *Trichomanes pinnatum* (Kupper, 1906; Goebel, 1928–1933). *Asplenium oceanicum* is of particular interest because it exhibits a frond dimorphism. The stoloniferous fronds that are capable of initiating buds are devoid of leaflets, whereas the photosynthetic fronds bear pinnae (Kupper, 1906).

A matter of concern has been how these shoots are initiated at the apex of the parent leaf and the nature of their relationship to the meristem of the parent leaf from which they arise. It is well established that the bud is initiated from the leaf apex well before the leaf has reached its full length and contacted the ground. Hence a certain amount of intercalary growth must take place along the rachis axis to extend the stolon. The early inception of the shoot bud convinced Goebel (1928–1933) to argue that such apical buds should not be called adventive. Their early inception on the leaf apex before it contacts the soil suggests that they are a fundamental part of the plant's organization, not really an abnormality as the term "adventive" would suggest.

There is general agreement that the apical initiation of the bud is from the leaf's apical meristem. However, there are differences of opinion as to whether the bud arises early on from the apical cell of the leaf (Kupper, 1906; McVeigh, 1934) or later from the marginal meristem (Kupper, 1906; Yarbrough, 1936; Beck, 1982; Rösch, 1982). Given that a more modern perspective is less concerned with cell lineages (Hagemann, 1964; McAlpin and White, 1974; Kaplan, 1977; Kaplan and Hagemann, 1991), the actual source of the meristem is less significant than what that inception means in terms of organogenesis and the degree of definition of fern organs.

While there have been arguments about the histological locus of the bud shoot apex, no one has contended that bud inception at the leaf apex represents a direct transformation of the fern leaf into a shoot. It is well known that fern leaves exhibit a widespread capacity to initiate shoots from their surfaces (McVeigh, 1937; Troll, 1939; Marchal, 1965). Moreover, Troll (1939) has argued that the locus of such leaf-borne buds is correlated with the regions of the leaf that are meristematic for the longest time, i.e., the margin and leaf apex. Hence, the initiation of shoots from the apices of these stoloniferous fronds does not involve the direct transformation of a leaf into a shoot. Because the apex of the frond is the region of the leaf that remains meristematic for the longest time, it will, perforce, be the prime site of adventitious bud inception.

When buds develop from leaves, the root primordia on the bud are initiated before its leaf primordia (Figure 9A–B; Kupper, 1906; Goebel, 1928–1933; McVeigh, 1934; Yarbrough, 1936; Beck, 1982; Rösch, 1982). It has been reported that the leaf primordia on the bud do not arise from a shoot apex (Figure 9A–B; Kupper, 1906; McVeigh, 1934), but those

Figures 7–8. Comparisons of stoloniferous growth between an angiosperm, *Saxifraga flagellaris* (7), and a fern, *Camptosorus rhizophyllus* (8). In the case of *Saxifraga*, the stolon is an axillary shoot that grows plagiotropically and then turns upright, forming a distal daughter rosette. In *Camptosorus*, the elongated leaf initiates a shoot bud at its apex, which in turn gives rise to the new rosette. NR, new rosette; S, stolon or runner; SF, stoloniferous frond.

reports used the presence of an apical cell as the criterion for shoot apex presence. It has since been shown that apical cell definition can be a relatively delayed developmental event and hence not a valid indicator of meristem presence (Hirsch and Kaplan, 1974; Croxdale, 1976). A more valid criterion for shoot apex definition is the production of leaf primordia. The parallels between the stages of development of these stoloniferous buds and embryogenesis in ferns are striking (compare Figure 9 with Figure 4). In both cases the earliest products are the first root and leaf, and the histological definition of the shoot apex is delayed. This stimulated Yarbrough (1936) to refer to the buds in the walking fern *Camptosorus* as foliar embryos, a terminology that is not inappropriate given the similarities in their development with embryogenesis.

Vining Habit—A second striking flowering-plant convergence in ferns is the twining plant life form (Figure 1C, D). In angiosperms shoot twiners are phanerophytic plants whose shoots are not self-supporting but reach a favorable position in the forest canopy by winding around a free-standing plant (phorophyte or support plant) or object (Figure 1D). Twining angiosperms develop rapidly elongating searcher shoots that circumnutate their support as their mode of ascent. These searcher shoots grow initially by internodal elongation at the expense of leaf enlargement. Leaf expansion occurs at a slower initial rate than in either self-supporting species or root climbers (Figure 1D; French, 1977). Moreover, leaves only expand to their full size in the anchored part of the shoot (Figure 1D).

There are twining ferns in the tropical genera *Lygodium*, *Salpichlaena*, and *Phanerosorus*. They differ from angiosperm vines in that it is the frond (leaf) that is the climbing organ rather than the shoot. The degree to which such vining fronds converge both developmentally and behaviorally with the shoots of flowering plant vines is striking.

The best studied genus of vining ferns is *Lygodium*, specifically *L. japonicum* (Mueller, 1982a, b, 1983). *Lygodium* grows in the forest by fronds that ascend their support to a height of 10–20 meters. The adult plant of *Lygodium japonicum* consists of a subterranean, plagiotropic, dorsiventral rhizome producing its indeterminate fronds in a distichous phyllotaxis (with a divergence of c. 35 degrees) from its dorsal surface and roots from its ventral surface (Figure 10; Mueller, 1982a). The rhizome branches dichotomously (Figure 10; Mueller, 1982a). Following their initiation from the shoot apex, fronds initially grow erect and the individual leaves push up through the soil surface before beginning their twining growth (Figure 11). As they grow upward they continue to initiate lateral leaflets from the leaf apical meristem (Figures 11, 12). As in other ferns, the persistence of apical meristematic activity in the frond is expressed by the persistence of a hyponastically coiled crozier at its apex (Figures 11–13).

The elongating frond of *Lygodium* resembles a twining angiosperm shoot not only in its spiral, circumnutating growth but also in the precocious elongation of its rachis "internodes," and the corresponding delay in the enlargement of its leaflets (Figures 12, 13). The leaflets do not become fully expanded but instead extend only the first two pinnules before the apex of each leaflet becomes arrested as a dormant crozier (Figure 14), forming what has been called a leaf bud (*Blattknospe*; Troll, 1939). Such leaf buds are not shoot buds like those initiated from frond surfaces in the stoloniferous species. They are simply apices of the leaflets which stop their growth (Figure 14), but retain the capacity to resume growth should the meristem at the tip of the rachis die or be removed (Figures 15–17; Mueller, 1983). Their foliar nature is indicated by their dorsiventrality and the fact that their plane of flattening is in the plane of dorsiventrality of the leaf as a whole (Figures 14, 15). Once stimulated to resume growth, such pinna apices continue to initiate lateral pinnules and the leaflets exhibit the same indeterminate growth as the parent rachis axis (Figures 15–17).

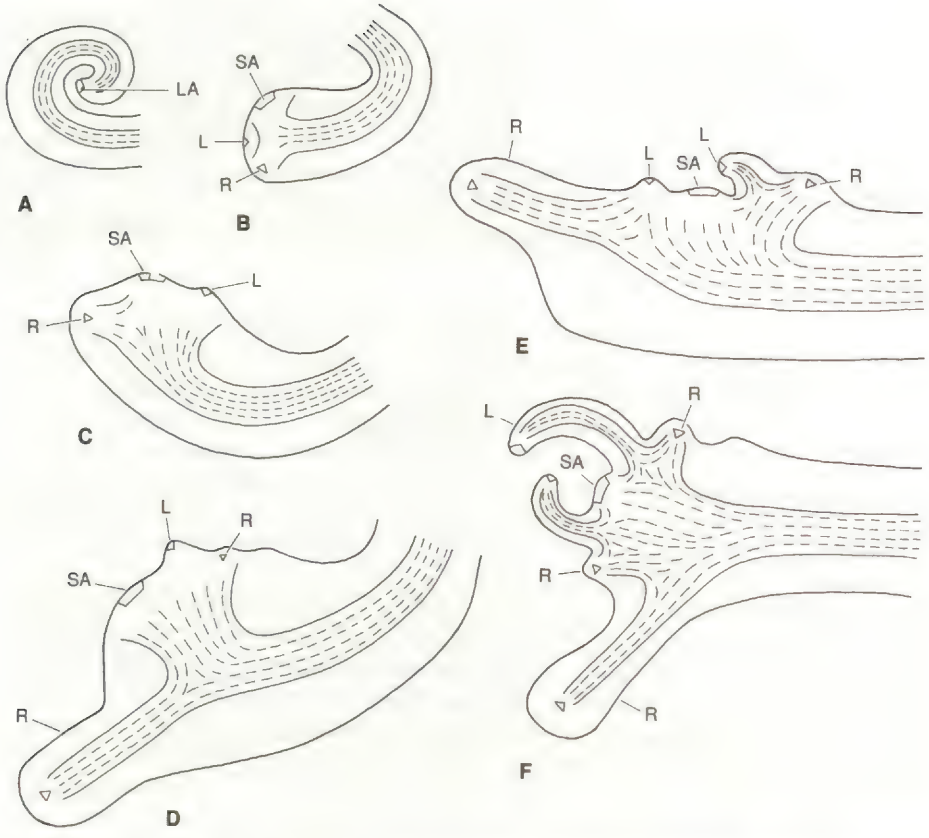


Figure 9. Median longisections of leaf apices of the fern *Asplenium sandersonii*, illustrating stages of development of a shoot from the apex of its stoloniferous frond.—A. Median longisection of frond apex. —B–F. Stages of bud development, including leaf and root inception, resembling the development of the fern embryo. L, leaf; LA, leaf apex; R, root; SA, shoot apex. [Redrawn with permission from Rösch (1982).]

Leaf buds are also found in the other climbing fronds in the genus *Phanerosorus*, and in the straggling, spreading fronds in species of *Dicranopteris* (Gleicheniaceae) and *Histiopteris* (Dennstaedtiaceae). Whereas the growth form of the climbing fronds in *Lygodium* and *Phanerosorus* is monopodial with leaf buds occurring at the apices of lateral pinnae (Figures 12, 13), pinna growth in Gleicheniaceae more typically is dichasial-sympodial with a leaf bud (arrested crozier) at the tip of each sympodial segment (Figure 18A). In *Dicranopteris linearis*, for example, each successive leaf bud terminates the next order of leaflet branching (Figure 18A). Like the leaf buds of *Lygodium*, those in *Dicranopteris* have the ability to resume frond growth if the dominant frond meristem is damaged.

The development of these leaf buds in climbing, spreading fern fronds is a remarkable feature convergent with shoot branching, but the details of the degree of the convergence are even more remarkable. In the genera *Histiopteris* (*H. incisa*) and *Dicranopteris*, leaf buds are developed at the tip of each frond segment. The basal-most pinnules of the adjacent pinnae are reduced and lobed in comparison with the more distal pinnules and function as stipular bud scale analogs, enclosing and presumably protecting the leaf bud at each pinna apex (Figure 18B, C).

Figures 10–14. Morphology of the shoot and developing leaves of the climbing fern, *Lygodium japonicum*. —10. Dichotomously branched, plagiotropic rhizome (stem) showing leaves in a distichous phyllotaxis on the dorsal surface and roots emerging from the ventral surface.—11. Young fronds with circinate vernation emerging from soil, i.e., from surface of subterranean rhizome like that shown in Figure 10.—12. Circumnutating leaf apex and rachis and expanding leaflets supported by a string.—13. Early, non-twining stage of leaf growth.—14. Mature rachis and leaf bud. C, crozier; L, leaf scars on rhizome; LB, leaf bud; LF, leaflet; R, root; RA, rachis axis. [Reproduced with permission of author and journal; from Mueller (1982a).]

Finally, while the greatly extended, indeterminate climbing frond of *Lygodium* would seem to be divergent, even for ferns, its homology with more conventional appendages is shown by the heteroblastic change in leaf form which *Lygodium* exhibits in the ontogeny of its shoot. Mueller (1982b) has shown that the juvenile shoot of *L. japonicum* begins with reduced dichotomously branched leaves (Figure 19) and proceeds through a series of intermediates that are larger in size and anisotomous in branching but still determinate, and finally produces the laterally branched, indeterminate climbing organs that are characteristic of the adult plant (Figure 19). By detailed comparative developmental investigations of each of these leaf types Mueller (1982a, b) was able to demonstrate the homology of the appendages in this leaf series and thereby show that the climbing leaf represents a divergence in relation to a specialized biological function and not an expression of a primitive, telomic-type of branch system.

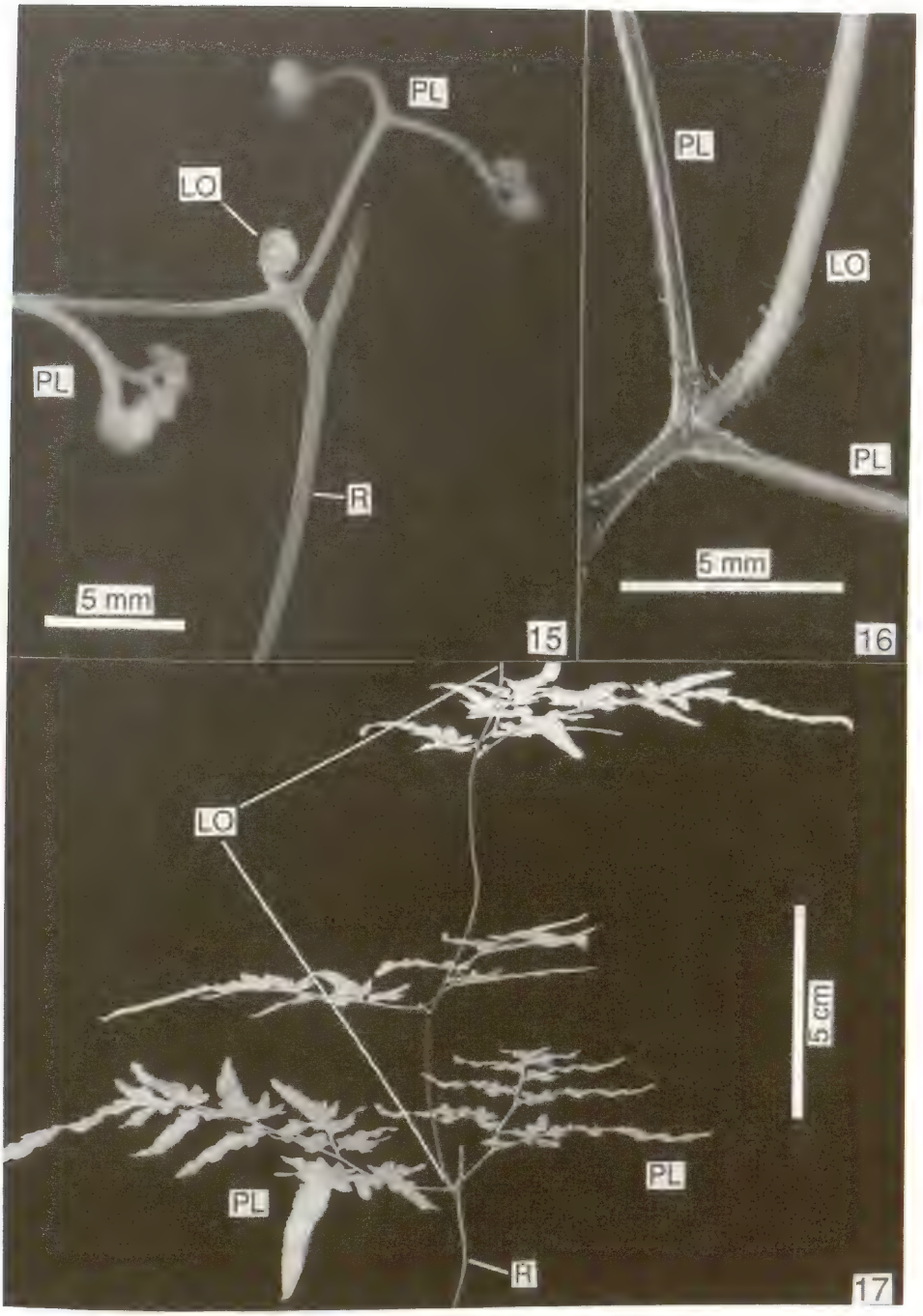
In the few angiosperm species that also exhibit elongated, pinnately dissected leaves with prolonged apical meristematic activity (*Guarea* and *Chisocheton* in Meliaceae), the juvenile shoots also begin with reduced, determinate leaf types with transitions to the larger, less determinate appendages (Steingraber and Fisher, 1986; Fisher and Rutishauser, 1990). Not only do these parallels between two unrelated plant groups underscore the fundamentality of this theme but they also affirm the leaf homology of the *Lygodium* frond.

Epiphytic Habit—The epiphytic life mode is characterized by structural and physiological specializations to cope with the problems of low moisture and nutrient availability (Benzing, 1990). Along with angiosperms, ferns are the major vascular plant group occupying this special habitat. Whereas orchid and bromeliad epiphytes have specializations in their roots or shoots, respectively, for adaptation to the epiphytic life mode, in ferns, as expected, structural specialization involves principally their leaves. For example, in the bird's nest fern *Asplenium nidus*, the rosette shoot is attached to its phorophyte (support plant) by its shoot-borne roots. The erect growing shoot bears long, lanceolate fronds in a helical phyllotaxis (Figure 20). By this mode of shoot construction the plant as a whole serves as its own flower pot, catching debris, accumulated humus, and moisture. The shoot-borne roots of *A. nidus* grow into this debris in the center of the rosette and absorb water and minerals (Figure 20).

This mode of humus accumulation is enhanced by specializations in the epiphytic shoot of the polypodiaceous genus *Aglaomorpha*. This species bears conventional pinnatifid fronds whose bases are lobed and where the central surface is broader than the marginal lobes (Figure 21). Such a regional leaf specialization enables the accumulated humus to be retained against the substratum and at the same time exposes an enlarged blade surface for assimilation (Figure 21).

This dual function of nutrient catchment and photosynthesis is expressed by a leaf dimorphism in the epiphytic genus *Drynaria* (*D. rigidula*, *D. quercifolia*, and *D. propinqua*). The plagiotropic rhizome of *Drynaria* initiates, in alternating series, mantle leaves and sporophyll-foliage leaves (Figures 22, 23). The two leaf types differ markedly in their morphology, mode of growth, function, and developmental fate. The foliage leaf is a typical, petiolate-pinnate photosynthetic organ, bearing sori on its abaxial surface (Figure 23). Because they show greater growth on their adaxial than abaxial surfaces, the foliage leaves stand away from the substrate (Figure 22). The foliage leaves abscise from the shoot after they die.

By contrast, the mantle leaves are much shorter, sessile, and pinnately lobed (Figure 23), but they grow erect, even appressed to the substrate because of greater growth on their abaxial than adaxial sides (Figure 22) and persist on the plant after they have died and lost their chlorophyll. Moreover, the mantle leaves have fewer stomata on their surfaces than the foliage leaves and exhibit a marked thickening of their lateral veins in the form of distinct



Figures 15–17. Stages in outgrowth and development of a leaf bud on the climbing frond of the fern *Lygodium japonicum*.—15. Leaf bud outgrowth and expanding pinnules, 7 days after removal of the crozier above the youngest (2 mm wide) pinna below it.—16. Original rachis, pinna stalk and base of leaf bud ramification.—17. Mature ramification (from leaf bud) with morphology identical to the parent leaf axis. LO, leaf bud outgrowth; PL, pinnule; R, original rachis. [Reproduced with permission of author and journal from Mueller (1983).]

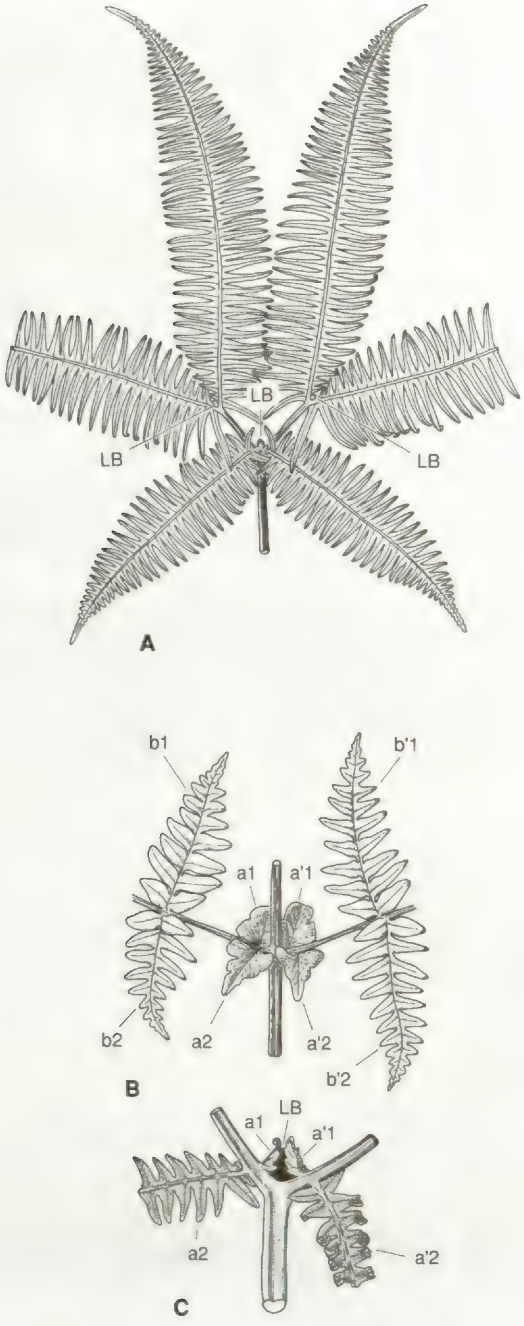


Figure 18. Leaf buds and their protection in fronds of ferns.—A. Frond of *Dicranopteris linearis* (Gleicheniaceae) showing dichasially branched frond with arrested croziers at the apices of the successive blade branch orders. —B. Detail of leaf bud on frond of *Histiopteris incisa* (Dennstaedtiaceae) showing lobed, stipule-like basal pinnules which protect the leaf bud. —C. Detail of leaf bud protection in *Dicranopteris linearis* showing the leaf bud protective leaflets, which are situated opposite the normally developed leaflets. a1, a2, basal pinnules; b1, b2, next higher set of pinnules; LB, leaf bud, arrested crozier. [Reproduced with permission from Troll (1939).]

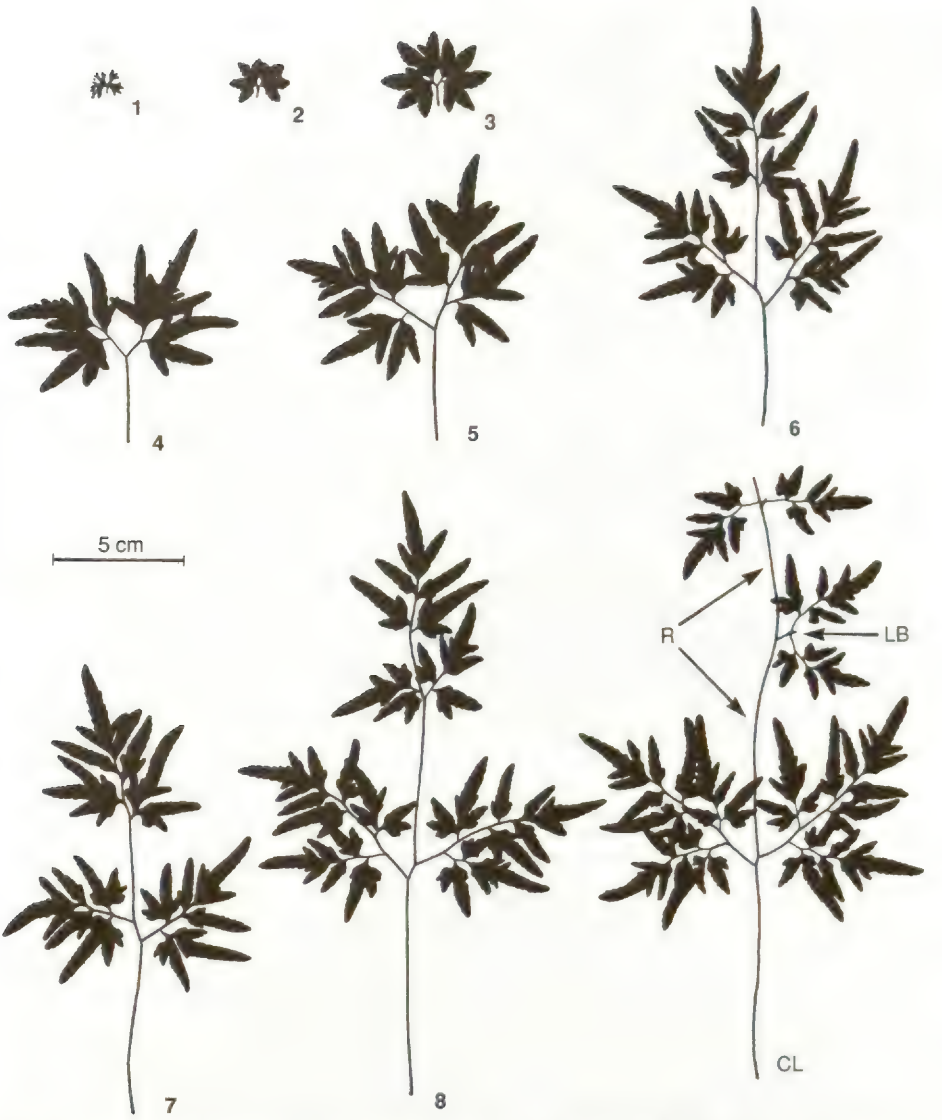


Figure 19. Heteroblastic leaf series in the climbing fern *Lygodium japonicum*. Primary leaves numbered 1–8 in order of appearance on the branched shoot system. Only the basal portion of the climbing leaf is shown. CL, climbing leaf; LB, leaf bud; R, rachis axis. [Reproduced with permission from Mueller (1982b).]

ribs (Figure 23). Hence the mantle leaves are specialized for humus accumulation and retention as well as providing a shaded locus for root development in this epiphytic habitat, whereas the foliage leaves function in assimilation and spore production and dispersal.

Such a leaf structure-function dimorphism is displayed even more dramatically in the staghorn fern *Platycerium* (Figure 25). The shoot of *Platycerium* consists of a plagiotropic, dorsiventral rhizome producing leaves in a distichous phyllotaxis from its dorsal surface (Figures 24, 26, 28, 29) and roots in profusion from its ventral side (Figure 27). Depending upon the species (Hoshizaki, 1972), the shoot produces, in irregularly alternating se-

quences, two contrasting leaf forms similar to *Drynaria* (Goebel, 1928–1933; Awan and Rao, 1981), so-called base fronds or mantle leaves and fertile fronds or foliage leaves. The mantle leaves are sessile, reniform, asymmetric organs that grow tightly appressed to the substrate and persist on the shoot after they have died (Figures 24, 26–29). The foliage leaves tend to be petiolate and dichotomously branched, and bear the coenosori on their abaxial surfaces. Furthermore, they are pendulous because of greater growth on their adaxial than abaxial surfaces (Figures 24–27). Like the fertile fronds of *Drynaria*, those of *Platycerium* abscise after their death (Figure 29). Because of their persistence one therefore sees a greater aggregation of mantle than foliage leaves on a shoot segment (Figures 24, 26, 29). *Platycerium* also proliferates shoots from its roots (Richards et al., 1983) to such an extent that a large specimen looks like an aggregation of many shoots (Figure 25).

Some species of *Platycerium* form only two distinct leaf types (e.g., *P. vassei* and *P. ellisii*), whereas others (*P. willinckii*) form a third type, a pocket leaf, which is intermediate between foliage and mantle leaves (Figure 28; Hoshizaki, 1972). Like the mantle leaves, the pocket leaves persist on the shoot, but like the foliage leaves, they do not grow so tightly appressed to the substrate (Figure 28). Thus in a large specimen the pocket leaves appear like a crown at the distal end of the shoot (Figure 28) and may play a more significant role in nutrient accumulation than the mantle leaves.

Some workers (Goebel, 1928–1933) have suggested that the mantle leaves, because of their thick, spongy texture, store water in the relatively dry habitat in which these epiphytes grow. However, Froebe and Strank (1981) have shown by comparative drying experiments that the mantle leaves of *P. bifurcatum* and *P. alcicorne* lose water at the same rate as other leaves. Rather the principal functions of such mantle organs would appear to be humus accumulation, provision of a dark, moist environment for root growth, and possibly also provision of heat insulation for the roots (A. Smith, pers. comm.).

Floating Aquatic Habit—Another specialized habitat that ferns share with flowering plants is water (Figure 1G, H). In both cases we are dealing with groups of vascular land plants of which certain members are presumed to have secondarily invaded the aquatic habitat. Angiosperms exhibit a greater diversity of aquatic life forms, but there are also submerged (*Ceratopteris*), floating (*Ceratopteris*, *Azolla,* and *Salvinia*), and amphibious ferns (e.g., Marsileaceae) in this habitat.

In a parallel to the marked reduction in the plant body shown by floating aquatic angiosperms such as *Lemna*, *Wolffia,* and *Wolffiella* (Sculthorpe, 1967), the floating aquatic fern genera show a similar reduction in their vegetative structure. However, despite this reduction they exhibit the same Bauplan as the other fern taxa and likewise show the elaboration of the leaf as the major functional unit.

The heterosporous floating aquatic fern *Salvinia* exemplifies this theme of fern morphology. The shoot of *Salvinia* consists of a plagiotropic axis bearing two different leaf types: large, dorsiventral, folded floating leaves and submerged, peltately dissected, submerged leaves (Figure 30). *Salvinia* has no roots. Hence what looks like a root is actually a leaf homologue consisting of up to approximately ten linear segments radiating from a central petiole (Figure 30A, C). Each of the leaflets is covered with trichomes, which presumably increase the surface area for water and ion uptake (Figure 30C). The shoot of *Salvinia* branches by forming extra-axillary shoot buds between the submerged leaf and closest floating leaf (Croxdale, 1978).

The leaf homology of the submerged appendage in *Salvinia* is indicated by the fact that it arises exogenously from the shoot apical meristem in a regular phyllotactic sequence with the floating leaves (Croxdale, 1978). Furthermore, the submerged leaflets are the sites of

Figure 20. Apical view of the rosette shoot of the epiphytic bird's nest fern *Asplenium nidus*. Detritus, debris and moisture accumulate in the center of the rosette where shoot-borne roots grow into it and take up water and mineral elements. The abundant hairs in the center of the rosette enclose and protect the apical meristem and young leaves.

sorus development (Figure 30C). Thus sporangia in *Salvinia* are leaf-borne just as they are in other ferns. Croxdale (1979) has also demonstrated developmentally that in *Salvinia* it is the abaxial rather than the adaxial surface of the floating leaves that is folded and is oriented toward the incident light (Figure 30A, C).

Figure 21. Foliage leaf (trophophyll) T and combination trophophyll sporophyll (TSp) in the polypodiaceous epiphyte, *Aglaomorpha meyeniana*. The base of the blade in both organs is widened to catch debris (WB). The sporophyllic organ differs from the foliage leaf in having fertile pinnae (FP) bearing sporangia at its distal end.

The phyllotactic pattern of *Salvinia* is distinctive. Each pair of floating leaves and a single submerged leaf form a triad, appearing to be inserted at a single node (Figure 30C). However, recent developmental investigations reveal that the phyllotactic pattern is subtle and more complicated. The leaves of each triad are initiated successively with the submerged leaf

Figures 22–25. Comparative leaf and shoot morphology in the polypodiaceous holoepiphytes *Drynaria* and *Platycerium*.—22. Whole plant and habit of *Drynaria rigidula* growing in a forest in New Caledonia. Shoot exhibits a conspicuous difference between mantle and foliage leaves. (Photo by D. M. Frame.)—23. Comparison of mantle and foliage leaves in *Drynaria rigidula*. Mantle leaf is sessile, persistent and pinnately lobed, whereas the foliage leaf is larger, pinnately dissected and distinctly petiolate.—24. Young plant of *Platycerium* sp. showing its clear delineation into appressed mantle leaves and pendant photosynthetic fronds.—25. Older and larger plant of *Platycerium bifurcatum* at the Fairchild Tropical Garden. The abundance of shoots suggests these are all root-borne shoots. FL, foliage leaf; FP, foliage leaf petiole; ML, mantle leaf.

Figures 26–29. Details of shoot morphology and organization in species of the epiphytic fern *Platycerium*.—26. Dorsal view of a young shoot of *P. bifurcatum* showing clear differences in morphology and growth mode of mantle and foliage leaves. Both appendages are initiated from the dorsiventral rhizome in a distichous phyllotaxis displaced toward the dorsal surface.—27. Ventral view of shoot from Figure 26 showing a profusion of roots initiated from the ventral side of the rhizome and enclosed by the mantle leaves. —28. Shoot of *P. willinckii*, showing foliage leaves, mantle leaves, and pocket leaves, with the latter being intermediate in morphology between foliar and mantle appendages.—29. Detail of phyllotactic arrangement on dorsal surface of shoot axis of *P. bifurcatum*, showing scars of abscised foliage leaves. FL, foliage leaf; FLS, foliage leaf scar; ML, mantle leaf; PL, pocket leaf; R, roots.

appearing first followed by the two floating leaves in a counterclockwise dorsal direction on the right side of the shoot and in a clockwise dorsal direction on the left side (Figure 30B; Croxdale, 1978). Interestingly, once all three members of a triad have been initiated, the direction of leaf expansion is in the reverse sequence of their initiation; i.e., the dorsal-most floating leaf enlarges first, followed by the lateral and ending with the submerged leaf (Figure 30B). Despite these phyllotactic idiosyncrasies, *Salvinia* is the most marked example of habitual anisophylly we know of in the plant kingdom (Troll, 1937; Croxdale, 1978, 1979, 1981). To date there are no clues as to what causal factors may be controlling this striking leaf dimorphism in *Salvinia*.

While *Salvinia* would seem to be very idiosyncratic among ferns, an analysis of its closest living relative, the floating aquatic *Azolla filiculoides*, indicates some more conventional connections. Like *Salvinia*, *Azolla* has a plagiotropic shoot bearing leaves in a distichous phyllotaxis (Figure 30D and E). *Azolla* differs from *Salvinia* in its possession of a few shoot-borne roots (Figure 30D). While the roots undoubtedly serve to anchor and balance the plant and to take up water and mineral elements, it is probable that, like *Salvinia*, the leaves of *Azolla* also play a role in uptake.

In contrast to *Salvinia*, the leaves of *Azolla* consist of two lobes that are asymmetric and differ significantly in their dimensions and tissue distribution. The dorsal lobes (U1–U4 in Figure 30F) are thicker in the median plane, whereas the ventral lobes (L in Figure 30F) are thinner in that plane and broader in the transverse plane (Figure 30E, F). Since it is the abaxial side of the dorsal lobes that is oriented toward the incident light at the water's surface, like *Salvinia*, its photosynthetic tissue is concentrated on the abaxial rather than the adaxial side (Figure 30F). The dorsal lobe is also the site where the symbiotic, nitrogen-fixing cyanobacterium *Anabaena* resides in a roofed cavity on the adaxial surface. The ventral lobe, by contrast, is colorless and tapers into a pair of uniseriate, marginal wings, which Goebel (1928–1933) suggested might be the site of water and ion uptake (Figure 30F). He cited the uptake of methylene blue dyes in these ventral lobes as evidence for their nutrient absorption role. The reduced number of roots and the much larger submerged leaf lobe surfaces make it possible to view *Azolla* as a less specialized example of root reduction and correlative leaf development. Hence in the same way that *Drynaria* exhibits a leaf dimorphism for the two different functions carried out by the leaves of *Aglaomorpha*, *Salvinia* exhibits a leaf dimorphism to carry out the uptake and photosynthetic functions executed by the two halves of the leaves of *Azolla*. Furthermore, the root reduction in floating ferns could represent an evolutionary parallel to the root reduction observed in various Lemnaceae in angiosperms.

Reproductive Structure—Finally, an equally striking example of analogies in structure between the leaf components of ferns and the shoots of angiosperms can be seen in a comparison of spore-bearing organs in the two groups. In flowering plants sporangia occur on specialized leaf homologues, the sporophylls (stamen or microsporophyll and the carpel or megasporophyll), which, in turn, are borne in a specialized reproductive shoot, the flower. Flowers themselves are frequently aggregated into an inflorescence in the reproductive region of the shoot system. Ferns also bear their sporangia on leaf surfaces but are unique in combining both the feeding (trophophyllic) and spore-bearing (sporophyllic) properties into a single organ, the frond. In the majority of ferns, aggregates of sporangia (sori) are borne on the abaxial surface of the frond, but there are several genera that bear their sporangia either on specialized fertile fronds or on spatially segregated fertile pinnae.

The so-called fertile spike in the adder's-tongue fern *Ophioglossum petiolatum* (Figure 31) is an example of a fern reproductive structure that is reminiscent of either a strobilus borne at the end of a shoot, as in various clubmosses (Lycopsida), or a spadix-type inflo-

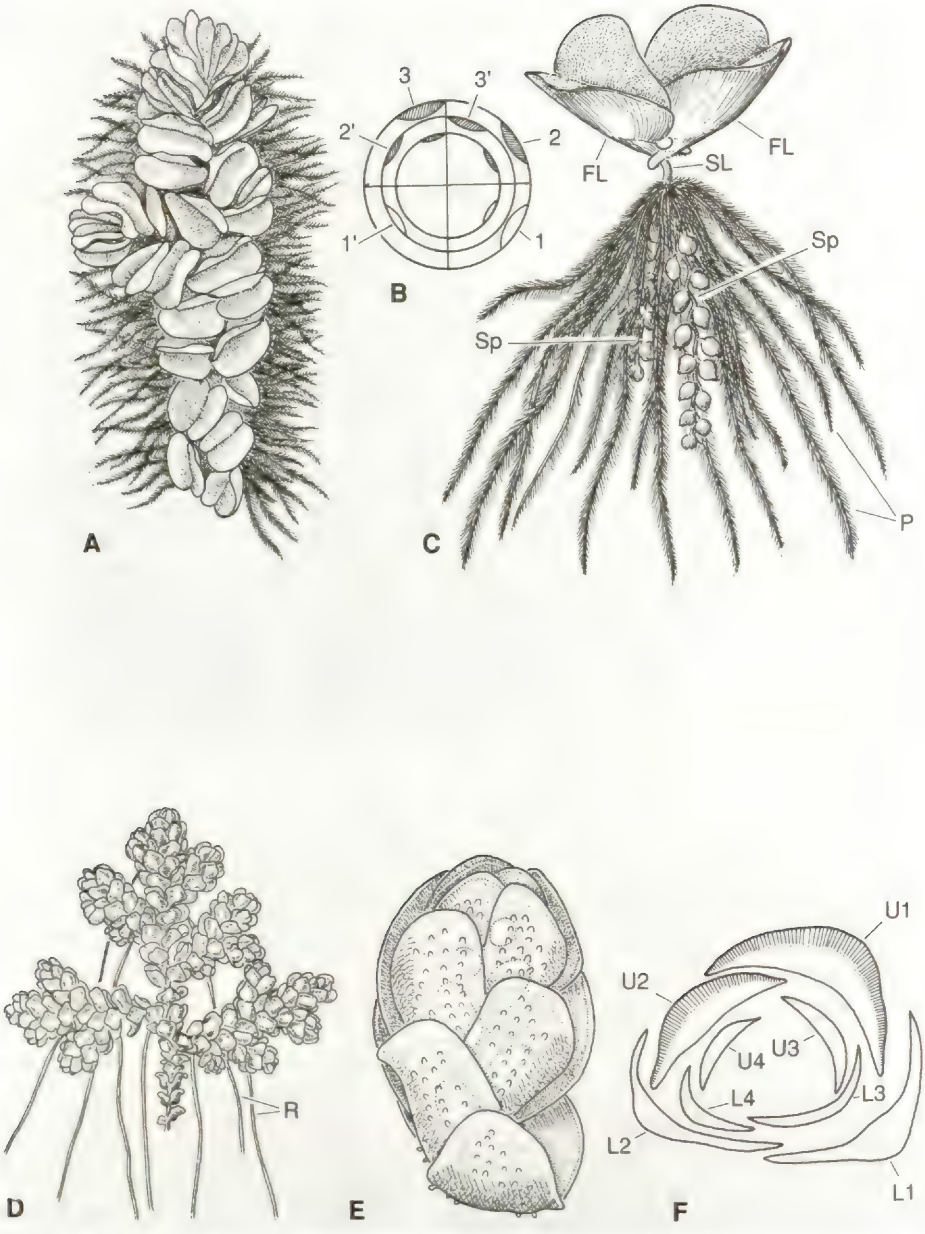


Figure 30. Morphology of floating aquatic fern genera *Salvinia* and *Azolla*. A–C. Shoot morphology of *Salvinia auriculata*.—A. Dorsal view of shoot.—B. Phyllotactic diagram showing sequence of leaf inception within each cycle from the data of Croxdale (1978). Hatched symbols are foliage leaves; open symbols, submerged leaves. —C. Lateral view of shoot showing differences in morphology between floating and submerged leaves at a node. D–F. Shoot of *Azolla filiculoides*.—D. Dorsal view of shoot system with roots.—E. Detailed view of bilobed leaves in a distichous phyllotaxis.—F. Shoot transection showing differences in transectional outlines of upper and lower leaf lobes. Striated lines indicate locus of photosynthetic tissues. FL, Foliage leaf; L1, L2, lower leaf lobes; P, pinnae; R, roots; SL, submerged leaf; Sp, sporangia; U1, U2, upper leaf lobes. [A–D reproduced with permission from Troll (1937); E–F from Goebel (1928–1933).]

rescence in Araceae in the angiosperms (Figure 32). In both of the aforementioned examples, we have a structure that is elevated on a stalk to lift the spores into the turbulent layer of the air in order to more effectively disperse them to a new site. In the case of *Ophioglossum,* we are actually dealing with an erect fertile pinna on a leaf homologue that converges with a seed plant reproductive shoot. How can we make such morphological deductions and hence relate this structure to the ground plan of ferns?

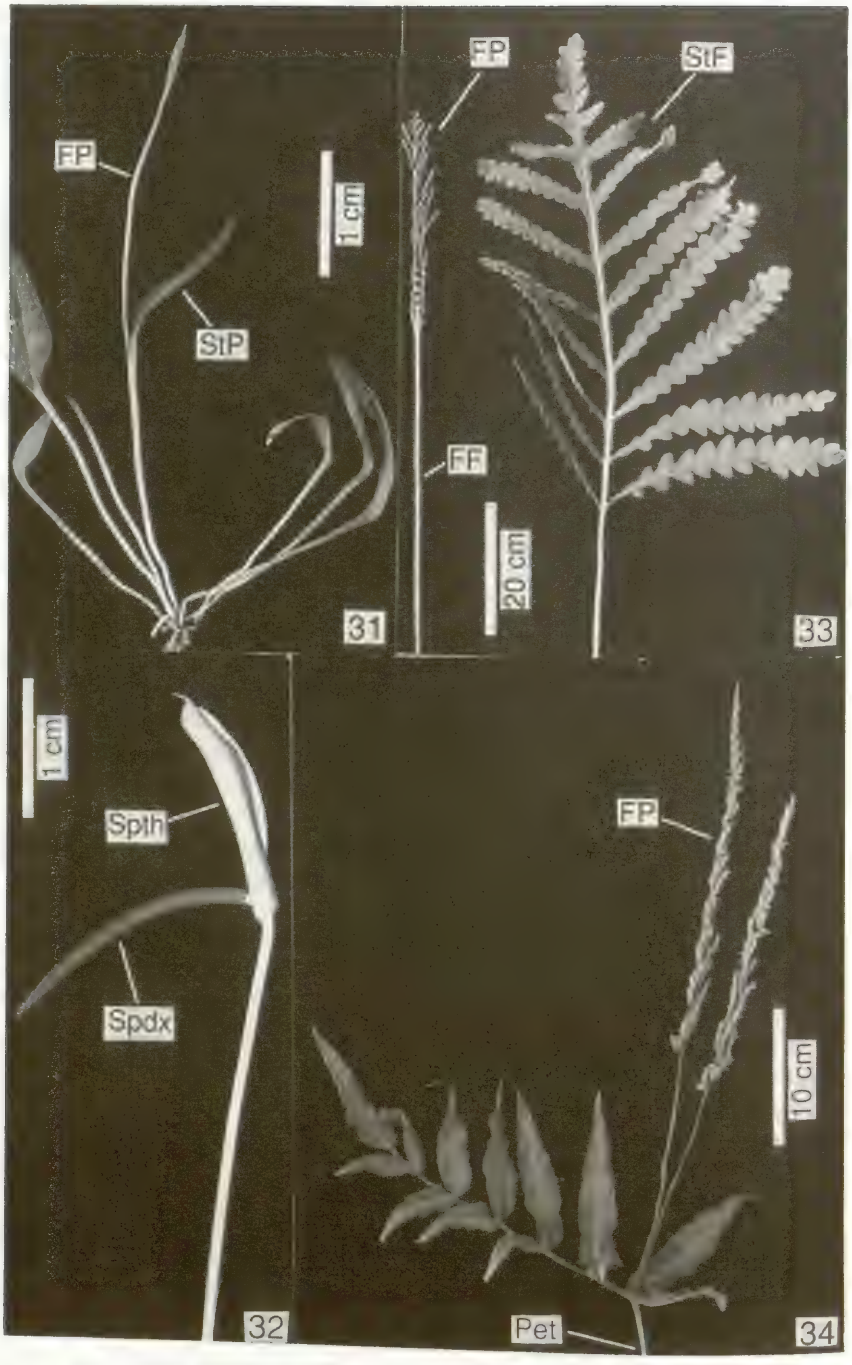
Contrary to the usual mode of fertile and feeding functions occurring on the same frond, there are several fern genera that have separate fertile and sterile (photosynthetic) fronds, for example, the sensitive fern, *Onoclea sensibilis*. This species begins its development initiating trophophylls exclusively. Subsequently, trophophylls bearing fertile pinnae are formed, followed finally by a dimorphism between fertile and sterile fronds (Figure 33). The latter are initiated in a regular, alternating sequence. Both fertile and sterile fronds are pinnate appendages, but the pinnae of the fertile frond bear sporangia exclusively, lack any growth in surface, and are erectly oriented in contrast to the horizontal orientation of the sterile pinnae (Figure 33).

Other fern species have fronds that are both trophophyllic and sporophyllic, but the division of labor is expressed at the level of pinnae. For example, the epiphytic fern *Aglaomorpha meyeniana* has fronds that are completely sterile and fronds that bear fertile pinnae distally and sterile leaflets proximally (Figure 21). In their restricted surfaces the fertile distal pinnae in *Aglaomorpha* resemble those on the fertile frond of *Onoclea* (compare Figure 21 with Figure 33). In this regard the genus *Osmunda* is of particular interest because frond morphology ranges from a complete frond dimorphism in *O. cinnamomea* (Figure 35), to several arrangements of fertile pinnae on a single frond in the other species. In the royal fern, *Osmunda regalis*, the fertile pinnae are terminal on the frond (Figure 36), whereas in *O. claytoniana* they are intercalary, being inserted between proximal and distal zones of sterile leaflets (Figure 37), reminiscent of the intercalary sporophyll distribution in shoots of *Tmesipteris tannensis* in Psilotaceae and *Lycopodium lucidulum* in Lycopsida (Gifford and Foster, 1989) and convergent with intercalary inflorescences in angiosperm genera such as *Callistemon* and *Ananas* (Weberling, 1981). *Osmunda vachelii,* on the other hand, has basally distributed fertile pinnae (Figure 38).

In *Anemia phyllitidis* (Schizeaceae), not only are the basal-most leaflet pairs fertile, like those in *Osmunda vechelii* (compare Figure 34 with Figure 38), but they are also erect, thereby separating the photosynthetic from the sporogenous pinnae for more effective spore dispersal and spatial differentiation of function. Like the single fertile spikes of *Ophioglossum* and *Botrychium*, not only do the two fertile pinnae in *Anemia* stand erect, but their sporangia-bearing pinnules are narrow and elevated on much longer petiolules for effective wind dispersal of their spores (compare Figure 34 with Figure 39A–B).

To go from the fertile leaf of *Anemia phyllitidis,* with its pair of erect lateral fertile pinnae and segregation of fertile and sterile leaf components (Figure 34), to *Ophioglossum petiolatum,* with the same distal sterile leaf region and proximal fertile spike (Figure 39B), is a relatively easy step. All it involves is the substitution of a single, median fertile leaflet for the pair of lateral fertile pinnae in *Anemia.*

Evidence for such homologies comes first from alternative leaf forms in *Botrychium lunaria*, where both the abaxial and adaxial leaf segments can be either sterile or fertile (Goebel, 1928–1933), indicating an homology between the two leaf blade regions. Moreover, the frond of *Ophioglossum palmatum* exhibits not only a median fertile leaflet associated with a unifacial petiolar symmetry (Figure 39C VIII–X) but also lateral fertile pinnae (spikes) associated with bifacial leaf sectors (Figure 39C II–VII). As seen in Figure 39C, the degree of approximation of the lateral fertile pinna pairs depends on how broad the bifacial sector is, or



Figures 31–34. Reproductive leaves and shoots in ferns and an angiosperm.—31. Shoot of *Ophioglossum petiolatum*, bearing a fertile leaf.—32. Spathe and spadix in flowering plant *Anthurium lechlerianum* (Araceae). —33. Frond dimorphism in *Onoclea sensibilis*.—34. Frond of *Anemia phyllitidis* bearing a pair of erect, fertile pinnae at the base of its blade. FF, fertile frond; FP, fertile pinna; Pet, petiole; Spdx, spadix; Spth, spathe; StF, sterile frond; StP, sterile pinna.

conversely, how widely separated the leaf margins are (Figure 39C II–VII). Where the leaf margins are closely approximated, then the fertile pinna will appear almost united (Figure 39C I, IX). If the leaf axis is unifacial when that single leaflet has its origin, then that leaflet will be median rather than lateral in insertion (Troll, 1939; Kaplan, 1970). Hence the fertile leaf in Ophioglossaceae can be regarded as a peltate organ in which a peltately dissected blade has been developed where the lateral leaflets have been suppressed and only the terminal and adaxial median leaflet opposite it are developed (Figure 39D–J; Troll, 1933, 1939). In most species of *Botrychium* and *Ophioglossum*, the terminal leaflet is normally sterile and the median adaxial pinna is fertile (Figure 39A–B, J).

In passing, it should be noted that Decker-Eisel and Hagemann (1978) have provided some evidence that the fertile pinna in *Ophioglossum pedunculosum* is actually lateral in origin from one of the two shanks of the marginal meristem, and therefore not truly peltate in morphology. According to these workers the homology of the fertile spike of *Ophioglossum* would be with *one* of the two lateral pinnae of *Anemia* rather than with a coalesced lateral pair. Unfortunately, the comparative developmental evidence needed to make this argument convincing was absent from their paper, and we therefore will have to have additional research before this issue can be resolved satisfactorily.

Thus, when we examine the morphological relationships of the seemingly aberrant frond in Ophioglossaceae on a broader, comparative basis, it is possible to understand it in the context of the basic Bauplan of fern shoots and fronds and to see how such an analogy with angiosperm shoots may have evolved structurally.

CONCLUSIONS

The foregoing examples of convergences between ferns and flowering plants show that the different ground plans (organization) of the two groups form consistent bases for their morphological diversification, a pattern of basic themes expressed in countless variations. Everything that flowering plants do with their whole shoots, ferns do with their individual leaves. Such convergences in function have also been the basis for the conclusion that fern fronds are really shoot homologues. But the critical comparative morphological analyses summarized in this paper provide more support for their convergent evolutionary nature than a homology in a structural and phylogenetic sense. These examples underscore the continuing need for critical morphological analyses before valid phylogenetic deductions can be drawn (Kaplan, 1977, 1984).

We have seen that the existence of such a distinctive ground plan does not preclude the evolution of special adaptations within each group. Even where a group of plants is morphologically unified by one or more consistent structural features, there can be remarkable variation in habit or physiognomy and in ecological life-history (Ashton, 1978; Groff, 1989; Tomlinson, 1970). In the same way that anatomical (cell to tissue) levels of organization are not predictive of morphological diversity (Kaplan and Hagemann, 1991), the basic morphological (organogenetic) levels of organization do not predict life form diversity. A particularly striking illustration of this concept is furnished by members of the family Cactaceae.

Shoots of cacti are generally characterized by primary thickening in either the pith or cortex regions of the stem, reduction of the leaf component to the leaf base, and axillary short shoots (areoles) bearing spines as leaf homologues. Despite this very distinctive shoot morphology, various species in subfamily Opuntioideae, for example, converge in overall architecture with trees, shrubs, and cushion plant life forms in other non-succulent dicotyledons (Rauh, 1979). Hence the very idiosyncratic shoot morphology that is diagnostic for Cactaceae has not precluded the evolution of marked habital variety. The striking convergences between the construction of ferns and flowering plants is also a prime illustration of the independence of life forms from morphology and underscores the fundamentality of this concept.

Figures 35–36. Distribution of sporangia in members of the fern genus *Osmunda*.—35. Dimorphic fronds in *O. cinnamomea*.—36. Terminal fertile leaflets in *O. regalis*. FF, fertile frond; FP, fertile pinna; StF, sterile frond; StP, sterile pinna.

It should also be stressed that such comparative morphological analyses and their evolutionary interpretation represent a process of reasoning after the fact. We can look within a group of organisms and derive after careful study a concept of their basic organization by comparison of their morphological variants. However, the ground plan we describe remains a human concept, an abstraction reflecting what morphological evolution within the group has thus far accomplished. Tomorrow, evolution may create from within our group a new form that violates our rules. Thus we emphasize the concept of developmental themes, reflecting what a group has "done" in terms of developmental morphology rather than the epistemologically more questionable concept of "developmental constraints," a term that seems to suggest absolute limits on what a group can do (Smith et al., 1985).

The problem with the concept of developmental constraint is that it can become a receptacle for our ignorance rather than a source of independent information about evolutionary limits set by a specific developmental system. With our limited knowledge of the develop-

Figures 37–38.—37. Intercalary distribution of fertile pinnae in the frond of *Osmunda claytoniana*.—38. Basal fertile pinna in fronds of *Osmunda vachelli*. FP, fertile pinnae; Pet, petiole; StP, sterile pinnae.

mental mechanisms in plants, especially at the organismal level, characteristics that do not seem to be the result of natural selection have been said to be the result of developmental constraints. Until we acquire a more profound understanding of the morphogenetic limits in higher plants we will not be able to discriminate the innate properties of a developing system from those that have been selected for in the group's evolutionary history. By using the concept of Bauplan, we have alluded to some innate developmental properties of the organism without implying that we know how they came into being.

While the structural relationships that we have characterized above have been fairly well worked out, the greatest gaps in our understanding are the functional or constructional considerations. Still largely unknown is the extent to which the fern analogies described really function like their angiospermic counterparts. For example, do the leaf lobes of *Azolla* really function like their angiospermic counterparts. For example, do the leaf lobes of *Azolla* really absorb nutrients? What is the role of the mantle leaves in *Drynaria* and in *Platycerium*? To answer such questions more effectively will require more combined field and laboratory studies of the physiological ecology of both ferns and their flowering plant analogs.

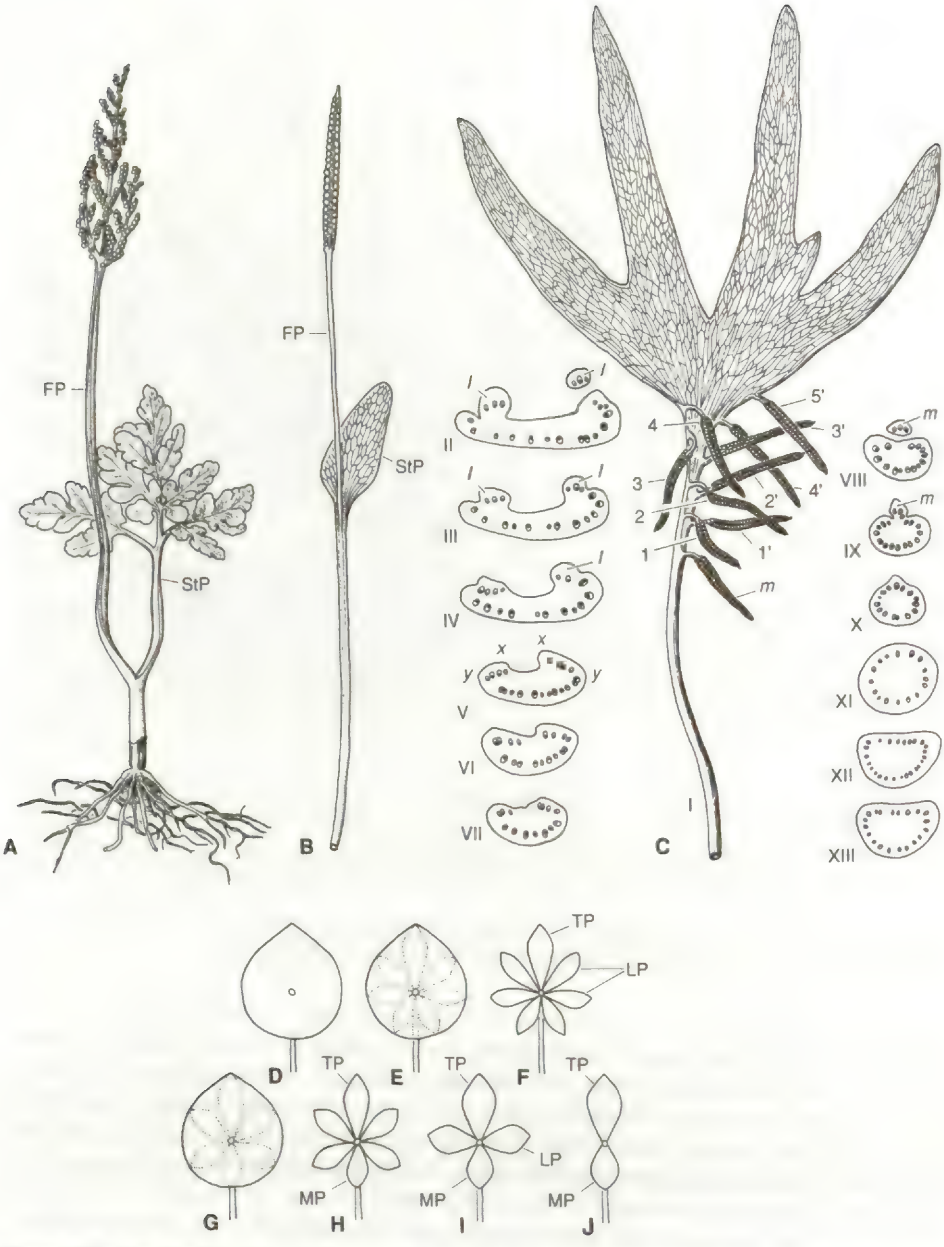


Figure 39. Fertile leaf morphology in members of the fern family Ophioglossaceae.—A. Shoot and aboveground leaf of *Botrychium matricariae*.—B. Fertile leaf of *Ophioglossum sp.*—C. Leaf of *Ophioglossum palmatum* bearing both lateral and a median fertile pinna. Transections II–XIII taken along length of leaf I to show relation of leaf transectional symmetry and mode of leaflet insertion.—D–J. Morphological relationship of fertile leaf of Ophioglossaceae to generalized peltate leaf and peltate dissected leaf (D and E). FP, fertile pinna; LP, lateral pinnae; MP, median pinna; StP, sterile pinna; TP, terminal pinna; l, laterally inserted fertile pinnae; m, medianly inserted fertile pinna; numbers in I give sequence of fertile pinnae. [Redrawn with permission from Troll (1939).]

LITERATURE CITED

Allsopp, A. 1965. Heteroblastic development in cormophytes. Pp. 1172–1221 *in* W. Ruhland (ed.), Handbuch der Pflanzenphysiologie. Springer, Berlin.

Ashton, P. S. 1978. Crown characteristics of tropical trees. Pp. 591–615 *in* P. B. Tomlinson and M. H. Zimmermann (eds.), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

Awan, B. and A. N. Rao. 1981. Developmental morphology of *Platycerium coronarium* (Koenig) Desv. Ann. Bot. 47: 805–816.

Beck, G. 1982. Zur Knospenbildung an den Blattspitzen der Ausläuferblätter von *Anemia rotundifolia* Schrad. Staatsexamen Arbeit. Univ. Heidelberg.

Benzing, D. H. 1990. Vascular Epiphytes. Cambridge Univ. Press, Cambridge.

Bierhorst, D. W. 1968. On the Stromatopteridaceae (fam. nov.) and on the Psilotaceae. Phytomorphology 18: 232–268.

_____. 1969. On *Stromatopteris* and its ill-defined organs. Amer. J. Bot. 56: 160–174.

_____. 1971. Morphology of Vascular Plants. Macmillan, New York.

_____. 1974. Variable expression of the appendicular status of the megaphyll in extant ferns with particular reference to the Hymenophyllaceae. Ann. Missouri Bot. Gard. 61: 408–426.

Bower, F. O. 1935. Primitive Land Plants. Macmillan, London (Reprint 1959, Hafner, New York).

Crotty, W. J. 1955. Trends in the pattern of primordial development with age in the fern *Acrostichum daneaefolium*. Amer. J. Bot. 42: 627–636.

Croxdale, J. G. 1976. Origin and early morphogenesis of lateral buds in the fern *Davallia*. Amer. J. Bot. 63: 226–238.

_____. 1978. *Salvinia* leaves I. Origin and early differentiation of floating and submerged leaves. Canad. J. Bot. 56: 1982–1991.

_____. 1979. *Salvinia* leaves II. Morphogenesis of the floating leaves. Canad. J. Bot. 57: 1951–1959.

_____. 1981. *Salvinia* leaves III. Morphogenesis of the submerged leaf. Canad. J. Bot. 59: 2065–2072.

Cutter, E. G. 1955. Experimental and analytical studies of pteridophytes XXIX. The effect of progressive starvation on the growth and organization of the shoot apex of *Dryopteris aristata* Druce. Ann. Bot. 19: 485–499.

Decker-Eisel, C. and W. Hagemann. 1978. Untersuchungen über den Ursprung der fertilen Fieder von *Ophioglossum pedunculosum*. Pl. Syst. Evol. 130: 143–155.

Eames, A. J. 1936. Morphology of Vascular Plants. Lower Groups. McGraw-Hill, New York.

Fisher, J. B. and R. Rutishauser. 1990. Leaves and epiphyllous shoots in *Chisocheton* (Meliaceae): a continuum of woody leaf and stem axes. Canad. J. Bot. 68: 2316–2328.

French, J. C. 1977. Growth relationships of leaves and internodes in viny angiosperms with different modes of attachment. Amer. J. Bot. 64: 292–304.

Froebe, H. A. and R. Classen. 1986. Das trialektische Konzept der Evolutionsmorphologie. Pp. 16–30 *in* H. A. Froebe and R. Classen (eds.), Grundsatzfragen der Pflanzenmorphologie. Aachen.

_____ and K. J. Strank. 1981. Zum wasserhaushalt von *Platycerium* Desv. Beitr. Biol. Pflanzen 56: 275–291.

Gifford, E. M. and A. S. Foster. 1989. Morphology and Evolution of Vascular Plants. 3rd. ed. W. H. Freeman, New York.

Goebel, K. von. 1928–1933. Organographie der Pflanzen. Dritte Aufl. Gustav Fischer, Jena.

Groff, P. A. 1989. Studies on whole-plant morphology. Ph.D. Dissertation, Univ. of California, Berkeley.

_____ and D. R. Kaplan. 1988. The relation of root systems to shoot systems. Bot. Rev. (Lancaster) 54: 387–422.

Hagemann, W. 1964. Vergleichende Untersuchungen zur Entwicklungsgeschichte des Farnsprosses. I. Morphogenese und Histogenese am Sprossscheitel leptosporangiater Farne. Beitr. Biol. Pflanzen 40: 27–64.

_____. 1967. Die Gestaltung als determinierendes Prinzip bei der Entwicklung des Pflanzenkörpers. Math. Naturwiss. Unterr. 20: 289–297.

_____. 1970. Studien zur Entwicklungsgeschichte der Angiospermenblätter. Bot. Jahrb. Syst. 90: 297–413.

_____. 1973. The organization of shoot development. Revista Biol. 9: 43–67.

_____. 1984. Die Baupläne der Pflanzen. Dritte Aufl. Privately Published, Heidelberg.

_____. 1992. The relationship of anatomy to morphology in plants: a new theoretical perspective. Int. J. Plant Sci. 153 (Suppl.): S38–S48.

_____ and D. R. Kaplan. 1989. The concept of marginal meristem in angiosperm leaf development. Amer. J. Bot. 76 (Suppl.): 36.

Hirsch, A. M. and D. R. Kaplan. 1974. Organography, branching and the problem of leaf versus bud differentiation in the vining epiphytic fern genus *Microgramma*. Amer. J. Bot. 61: 217–229.

Hoshizaki, B. J. 1972. Morphology and phylogeny of *Platycerium* species. Biotropica 4: 93–117.

Kaplan, D. R. 1970. Comparative development and morphological interpretation of "rachis-leaves" in Umbelliferae. *In* N. K. B. Robson, D. F. Cutler, and M. Gregory (eds.), New research in plant anatomy. Suppl. Bot. J. Linn. Soc. Lond. 63: 101–125.

_____. 1973. Comparative developmental analysis of the heteroblastic leaf series of axillary shoots of *Acorus calamus* L. (Araceae). Cellule 69: 251–290.

_____. 1977. Morphological status of the shoot systems of Psilotaceae. Brittonia 29: 30–53.

_____. 1980. Heteroblastic leaf development in *Acacia*: morphological and morphogenetic implications. Cellule 73: 135–203.

_____. 1984. The concept of homology and its central role in the elucidation of plant systematic relationships. Pp. 51–70 *in* T. Duncan and T. F. Stuessy (eds.), Cladistics: Perspectives on the Reconstruction of Evolutionary History. Columbia Univ. Press, New York.

_____ and W. Hagemann. 1991. The relationship of cell and organism in vascular plants. BioScience 41: 693–703.

Kupper, W. 1906. Über Knospenbildung an Farnblättern. Flora 96: 337–408.

Mabberley, D. J. 1987. The Plant-Book. Cambridge Univ. Press, Cambridge.

Marchal, M. 1965. Le Bourgeonnement épiphylle spontané des Fougères tropicales. Adansonia 5: 239–270.

McAlpin, B. W. and R. A. White. 1974. Shoot organization in the Filicales: the promeristem. Amer. J. Bot. 61: 562–579.

McVeigh, I. 1934. Vegetative reproduction in *Camptosorus rhizophyllus*. Bot. Gaz. 95: 503–510.

_____. 1937. Vegetative reproduction of the fern sporophyte. Bot. Rev. (Lancaster) 3: 457–497.

Mueller, R. J. 1982a. Shoot morphology of the climbing fern *Lygodium* (Schizaeaceae): General organography, leaf initiation, and branching. Bot. Gaz. 143: 319–330.

_____. 1982b. Shoot ontogeny and the comparative development of the heteroblastic leaf series in *Lygodium japonicum* (Thunb.) Sw. Bot. Gaz. 143: 424–438.

_____. 1983. Indeterminate growth and ramification of the climbing leaves of *Lygodium japonicum* (Schizaeaceae). Amer. J. Bot. 70: 682–690.

Rauh, W. 1979. Kakteen an ihren Standorten. 2nd Aufl. Paul Parey, Berlin.

Richards, J. H., J. Z. Beck, and A. M. Hirsch. 1983. Structural investigations of asexual reproduction in *Nephrolepis exaltata* and *Platycerium bifurcatum*. Amer. J. Bot. 70: 993–1001.

Rösch, D. 1982. Die Blattknospenbildung bei *Asplenium sandersoni* Hook. Staatsexamen Arbeit. Univ. Heidelberg.

Sculthorpe, C. D. 1967. The Biology of Aquatic Vascular Plants. St. Martin's, New York.

Seilacher, A. 1970. Arbeitskonzept zur Konstruktionsmorphologie. Lethaia 3: 393–396.

Smith, J. M., R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert. 1985. Developmental constraints and evolution. Quart. Rev. Biol. 60: 265–287.

Steingraber, D. A. and J. B. Fisher. 1986. Indeterminate growth of leaves of *Guarea* (Meliaceae): a twig analogue. Amer. J. Bot. 73: 852–862.

Tomlinson, P. B. 1970. Monocotyledons—towards an understanding of their morphology and anatomy. Advances Bot. Res. 3: 207–292.

Troll, W. 1933. Über die Blattbildung der Ophioglossaceen, insbesondere von *Ophioglossum*. Planta 19: 547–573.

_____. 1937. Vergleichende Morphologie der höheren Pflanzen. Band 1, Teil 1. Gebrüder Borntraeger, Berlin.

_____. 1939. Vergleichende Morphologie der höheren Pflanzen. Band 1, Teil 2. Gebrüder Borntraeger, Berlin.

_____. 1959. Allgemeine Botanik. Dritte Aufl. Ferdinand Enke, Stuttgart.

_____ and W. Rauh. 1950. Das Erstarkungswachstum krautiger Dikotylen, mit besonderer Berücksichtigung der primären Verdickungsvorgänge. Sitzungsber. Heidelberger Akad. Wiss. Math.-Naturwiss. Kl. 1950: 3–86.

Wardlaw, C. W. 1949. Further experimental observations on the shoot apex of *Dryopteris aristata* Druce. Philos. Trans., Ser. B 233: 415–451.

Weber, H. 1958. Konstruktionsmorphologie. Zoologische. Jahrb. Abt. Allg. Zool. Physiol. Tiere 68: 1–112.

Weberling, F. 1981. Morphologie der Blüten und der Blütenstände. Ulmer, Stuttgart.

Yarbrough, J. A. 1936. The foliar embryos of *Camptosorus rhizophyllus*. Amer. J. Bot. 23: 176–181.

Zimmermann, W. 1930. Die Phylogenie der Pflanzen. Gustav Fischer, Jena.

DISTRIBUTION OF ARCHITECTURES ACROSS A TAXONOMIC SPECTRUM

Francis Hallé[1]

ABSTRACT. Two basic concepts of architecture, the model and the reiteration, have much evolved since the 1970s, when they were introduced. The Architectural Model should no longer be considered as restricted to plants, for it features any fixed living being, plant or animal. Its construction mechanism should no longer be included in the model's definition, for the model is merely a global shape, having a high probability to be built by many different plant species, irrespective of the specific pathway used by each of them. As compared to fixed ones, free-moving animals are architecturally poor. The Reiteration process is a frequent, if not constant, feature of fixed living beings, animals or plants. Some tree species do not reiterate, whereas others do. The way reiterated complexes making up a tree crown are linked to each other is a new field of research enlightening the fact that most of the trees are colonies. The reiteration strategies could be used in systematics.

The discipline of plant architecture has greatly evolved since its beginning 20 years ago (Hallé and Oldeman, 1970; Hallé, Oldeman, and Tomlinson, 1978). The two basic concepts in architecture, the **Model** and the **Reiteration**, have been considerably developed. The purpose of my contribution is to present how these concepts have matured and how they can be used in identification and biosystematics.

THE ARCHITECTURAL MODEL

The architectural model is a stable character in a given plant species, although quantitative modifications can be induced by environmental changes. It remains stable during the course of ontogeny; however, in a few cases, we know of architectural mutations that can qualitatively modify the architecture (Hallé, 1978; Doebley, 1994).

In general, plant architecture has received scant attention from systematists except in a few cases (Stone, 1970a, 1970b; Hallé, 1967, 1971; Tomlinson and Esler, 1973; Guillaumet, 1973; Tomlinson, 1977, 1982, 1984; Temple, 1977; Philipson, 1978; Del Tredici, 1985; Fisher and Weeks, 1985). The integration of plant architecture into systematics depends on field observation and is not adapted to the herbarium or laboratory, where most taxonomy is still produced. However, it is widely used for field identification by foresters, local scientists, and villagers. Figures 1 and 2 demonstrate the breadth of architectural diversity in the family Loganiaceae and in the genus *Euphorbia* in Madagascar. Although those two examples were selected for their high degree of architectural diversity, it is usual for families and even genera to display several architectural models. Therefore, architecture has potential value as a systematic character at various taxonomic levels.

Figure 3 shows four examples of Massart's model (*Araucaria*, *Platanus*, *Diospyros*, and *Pycnanthus*) and conveys a new vision of the architectural model. These four trees are either monopodial (*Araucaria, Diospyros*) or sympodial (*Platanus*), and their sexuality is either terminal (*Araucaria*), lateral (*Pycnanthus*), or even cauliflorous (*Diospyros*). Nevertheless, they all belong to Massart's model, showing that the external manifestation of a given model results from differing developmental pathways in the constituent species. The same

[1]Laboratoire de Botanique, Institut Botanique, 163 rue A. Broussonet,, F-3400 Montpellier, France.

model is shared by a variety of plant species, and the specific expression of the model is called the "Architectural Unit" (Barthélémy et al., 1991). Furthermore, the taxonomic spectrum of architecture has been much enlarged. The concept of the model should no longer be applied in a restrictive sense to plants alone, for it can describe any fixed living being, plant or animal.

Figure 4 shows five architectural models already found in corals (Dauget, 1991, 1985). Although this investigation is still in its infancy, it is already clear that, as far as architecture is concerned, the divide between animals and plants is less relevant than the divide between *fixed* and *free-living* organisms. The fixed state imposes equivalent constraints and induces similar responses, which transcend the animal/plant division.

THE REITERATION

The process of reiteration, defined by Oldeman (1972, 1974), is the repetition of the architectural blueprint of the plant, or part of it. It is either a response to trauma (traumatic reiteration) or a normal component of plant ontogeny (adaptive reiteration). Adaptive reiteration is common but not universal in plants. *Araucaria muelleri* (Carr.) Brongn. & Gris. from New Caledonia (Veillon, 1978) is a rare example of a gymnosperm that always grows in strict conformity with its model. *Pandanus*, palms, and pioneer trees such as *Anthocleista* (Loganiaceae) or *Musanga* (Moraceae) are also examples of non-reiterating plants, meaning that they do not reiterate adaptively, only as a response to trauma.

Figure 5 shows that reiteration exists in corals; it exists also in other groups of fixed animals. Figures 6 and 7 show that trees in the tropical rain-forests are easily identified from a distance by their specific reiteration strategies. Although reiteration hides the architectural unit, it does not prevent identification even at the specific level. As a consequence of reiteration, a big tree—Oak, Mahogany, or Dipterocarp—is not an individual, but a colony of elementary individuals. Each reiterated complex is an individual.

Research is in progress to analyze relationships between the reiterated complexes that constitute a tree-crown. It is hypothesized that these relationships involve competition for water uptake, perhaps parasitism, and other factors.

LITERATURE CITED

Barthélémy, D., C. Edelin, and F. Hallé. 1991. Canopy architecture. Pp. 1–20 *in* A. S. Raghavendra (ed.), Physiology of Trees. Wiley, New York.

Caraglio, Y. and C. Edelin. 1990. Architecture et dynamique de croissance du platane *Platanus hybrida* Brot. (PLATANACEAE) (Syn. *Platanus acerifolia* (Aiton) Willd). Bull. Soc. Bot. France, Lett. Bot. 137: 279–291.

Cremers, G. 1975. Sur la présence de dix modèles d'architecture végétative chez les Euphorbes malgaches. Compt. Rend. Hebd. Séances Acad. Sci., Sér. D 281: 1575–1578.

_____. 1977. Architecture végétative de quelques espèces malgaches du genre *Euphorbia* L. Bull. Jard. Bot. Belg. 47: 55–81.

Dauget, J. M. 1985. La réaction aux traumatismes: comparaison entre les arbres et les coraux. Rev. Écol. 40: 113–118.

_____. 1991. Application of tree architectural models to reef-coral growth forms. Mar. Biol. 111: 157–165.

Del Tredici, P. 1985. The layered look: the humble bunchberry yields startling insights into tree architecture. Arnoldia 45: 19–22.

Doebley, J. 1994. Genetics, development, and the morphological evolution of maize. Pp 57-70 *in* P. C. Hoch and A. G. Stephenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard. (in press).

Edelin, C. 1990. The monopodial architecture: the case of some tree species from tropical Asia—Research Pamphlet no. 105. Forest Res. Inst. Kuala Lumpur

_____ and F. Hallé. 1985. Architecture et évolution chez deux genres d'arbres tropicaux: *Diospyros* et *Cordia*. Actes du 110° Congrès National des Sociétés Savantes, Montpellier, 1985. Section des Sciences, fasc. II, Biologie animale et Biologie végétale: 255–265. Comité des Travaux Historiques et scientifiques, Ministère de l'Education Nationale, Paris.

Fisher, J. B. and C. L. Weeks. 1985. Tree architecture of *Neea* (Nyctaginaceae). Geometry and simulation of branches and the presence of two different models. Bull. Mus. Natl. Hist. Nat., Sér. 4, Misc. 7: 385–401.

Guillaumet, J. L. 1973. Formes et développement des *Pandanus* Malgaches. Webbia 28: 495–519.

Hallé, F. 1967. Etude biologique et morphologique de la tribu des Gardéniées (Rubiacées). Mém. ORSTOM 22: 1–146.

_____. 1971. Architecture and growth of tropical trees exemplified by the Euphorbiaceae. Biotropica 3: 56–62.

_____. 1978. Architectural variation at the species level in tropical trees. Pp. 209–221 *in* P. B. Tomlinson and M. H. Zimmermann (eds.), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

_____. 1980. Vegetative architecture—Loganiaceae. Pp. 97–111 *in* A. Engler and K. Prantl (eds.), Die Natürlichen Pflanzenfamilien. Duncker et Humbolt, Berlin.

_____ and R. A. A. Oldeman. 1970. Essai sur l'architecture et la dynamique de croissance des arbres tropicaux. Collection de monographies de Botanique et de Biologie Végétale, 6. Masson et Cie, Paris.

_____, _____, and P. B. Tomlinson. 1978. Tropical Trees and Forests: An Architectural Analysis. Springer-Verlag, Berlin, Heidelberg, New York.

Oldeman, R. A. A. 1972. L'architecture de la végétation ripicole forestière des fleuves et criques guyanais. Adansonia 12: 253–265.

_____. 1974. L'architecture de la forêt guyanaise. Mém. ORSTOM 73: 1–204.

Philipson, W. R. 1978. Araliaceae: Growth forms and shoot morphology. Pp. 269–284 *in* P. B. Tomlinson and M. H. Zimmermann (eds.), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

Stone, B. C. 1970a. Observations on the genus *Pandanus* in Madagascar. Bot. J. Linn. Soc. 63: 97–131.

_____. 1970b. Morphological studies in Pandanaceae. II. The "coniferoid" habit in *Pandanus* sect. *Acanthostyla*. Bull. Torrey Bot. Club 97: 144–149.

Temple, A. 1977. Botanique—Ericaceae: Polymorphisme architectural d'une famille des régions tempérées et tropicales d'altitude. Compt. Rend. Hebd. Séances Acad. Sci., Sér. D 284: 163–166.

Tomlinson, P. B. 1977. Plant morphology and anatomy in the tropics: the need for integrated approaches. Ann. Missouri Bot. Gard. 64: 685–694.

_____. 1982. Chance and design in the construction of plants. Acta Biotheor. 31: 162–183.

_____. 1984. Vegetative morphology: some enigmas in relation to plant systematics. Pp. 49–66 *in* V. H. Heywood and D. M. Moore (eds.), Current Concepts in Plant Taxonomy. Systematics Association Special Publication, Vol. 25. Academic Press, New York.

_____ and A. E. Esler. 1973. Establishment growth in woody monocotyledons native to New Zealand. New Zealand J. Bot. 11: 627–644.

Veillon, J. M. 1978. The architecture of the New Caledonian species of *Araucaria*. Pp. 233–245 *in* P. B. Tomlinson and M. H. Zimmermann (eds.), Tropical Trees as Living Systems. Cambridge Univ. Press, Cambridge.

_____. 1980. Architecture des espèces néo-calédoniennes du genre *Araucaria*. Candollea 35: 609–640.

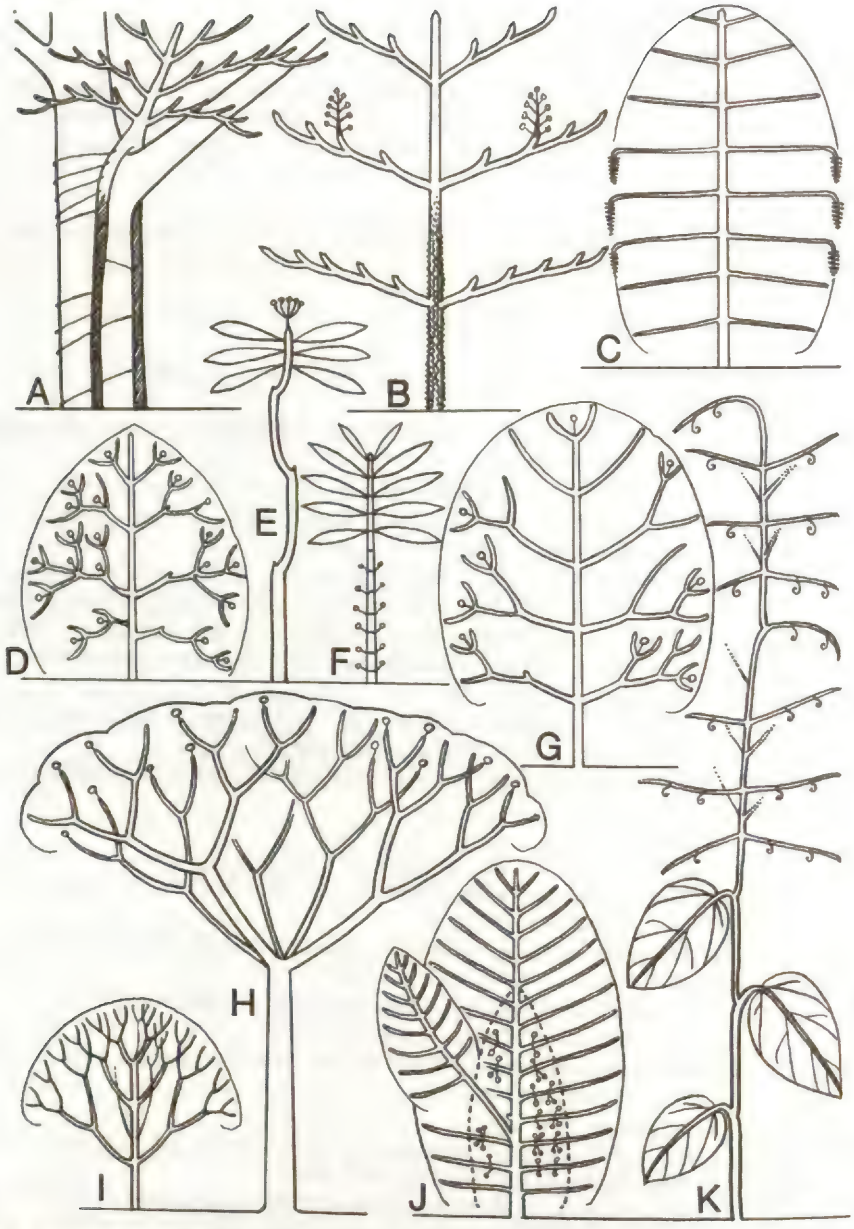


Figure 1. Architectural diversity in Loganiaceae (Hallé, 1980). A. *Fagraea berteriana* A. Gray: an epiphytic shrub of New Caledonia; PETIT's model. B. *Fagraea crenulata* Maingay ex Clarke: a tree 20 m tall, in swampy forests of Malaysia and Indonesia; FAGERLIND's model. C. *Fagraea racemosa* Jack. ex Wall.: a small tree of secondary forest throughout South East Asia; ROUX's model. D. *Neuburgia corynocarpa* (A. Gray) Leenh.: a rain-forest tree from New Caledonia; STONE's model. E. *Potalia amara* Aublet: a monocaulous shrub, less than 2 m high, in the rain forest of French Guiana; CHAMBERLAIN's model. F. *Mostuea brunonis* Didr.: a monocaulous shrub, less than 1 m high, in the Congolese rain forests; CORNER's model. G. *Anthocleista amplexicaulis* Bak.: a tree 8 m tall, in swampy forests of Madagascar; SCARRONE's model. H. *Anthocleista nobilis* G. Don: a tree up to 30 m tall, in the secondary forests of West Africa; LEEUWENBERG's model. I. *Logania imbricata* (Guill.) Steen. & Leenh.: an hemispheric-shaped shrub, 1 m high, in New Caledonia; architecture close to LEEUWENBERG's model. J. *Geniostoma ligustrifolium* A. Cunn.: a shrub 2 m tall, from New Zealand. K. *Strychnos* sp.: a rain-forest liane from French Guiana (Herbarium of Montpellier University, F.H. 2637).

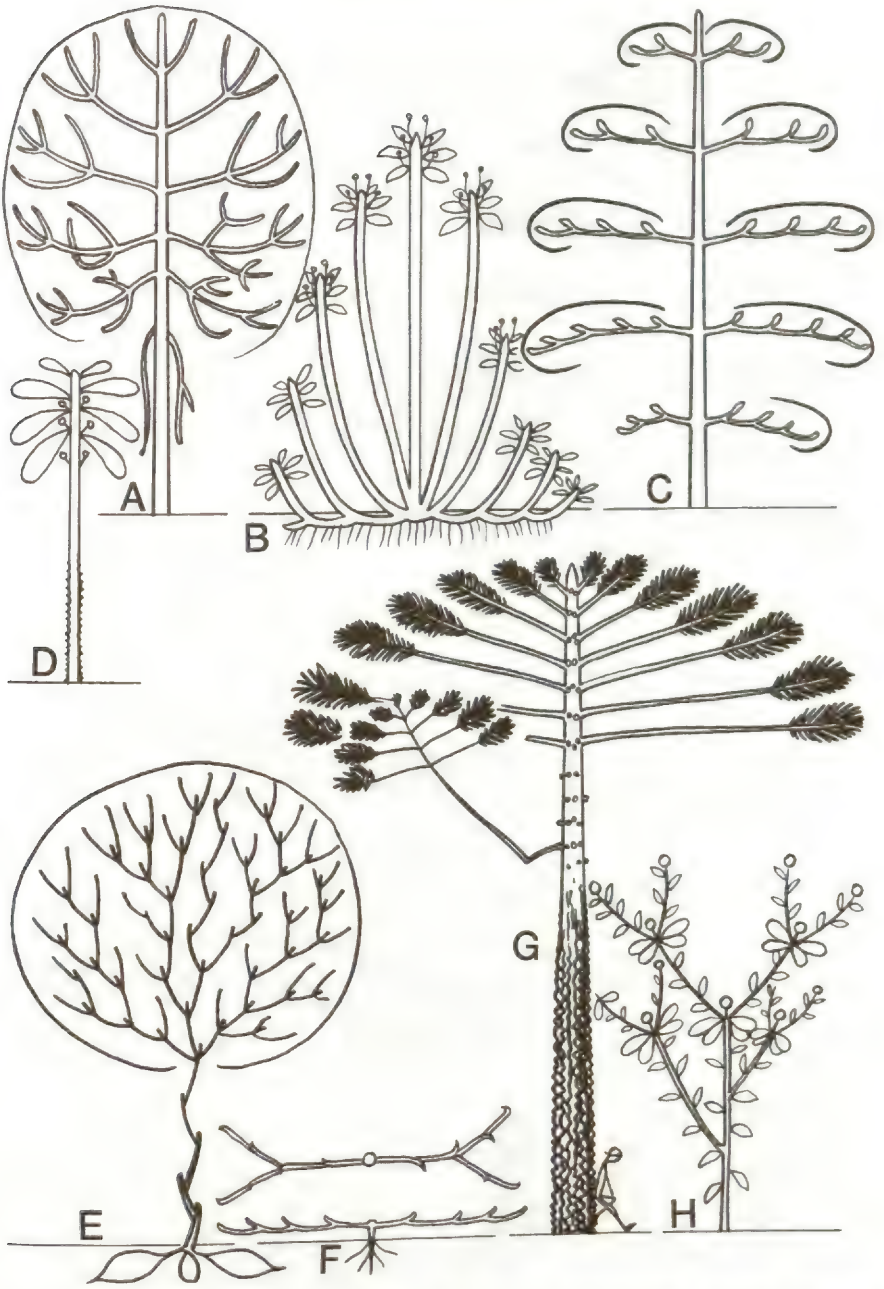


Figure 2. Architectural diversity among the species of *Euphorbia* L. in Madagascar (Cremers, 1975, 1977). A. *Euphorbia leucodendron* Drake: a small tree 4 m tall, from the South; RAUH's model. B. *E. milii* Desm. var. *breoni* (Nois.) Ursch & Leandri: an herb up to 3 m tall, from the Center (Antsirabe); TOMLINSON's model. C. *E. hedyotoides* N. E. Br.: a small tree 4 m tall, from the West (Bemaraha Plateau); AUBREVILLE's model. D. *E. lophogona* Lam.: a small woody plant less than 1 m tall, from the East (Fénérive, Fort Dauphin); CORNER's model. E. *E. intisy* Drake: a small tree up to 3 m tall, from the South (Tsivory); KORIBA's model. F. *E. prostata* Aiton; a widespread creeping weed, having an unnamed architecture. G. *E. enterophora* Drake: the highest of the Malagasy spurges, a tree up to 20 m tall, from the South-West (Zombitsy); MASSART's model. H. *E. emirnensis* Baker: an herbaceous spurge less than 1 m tall, from the Center (Ankaratra); LEEUWENBERG's model.

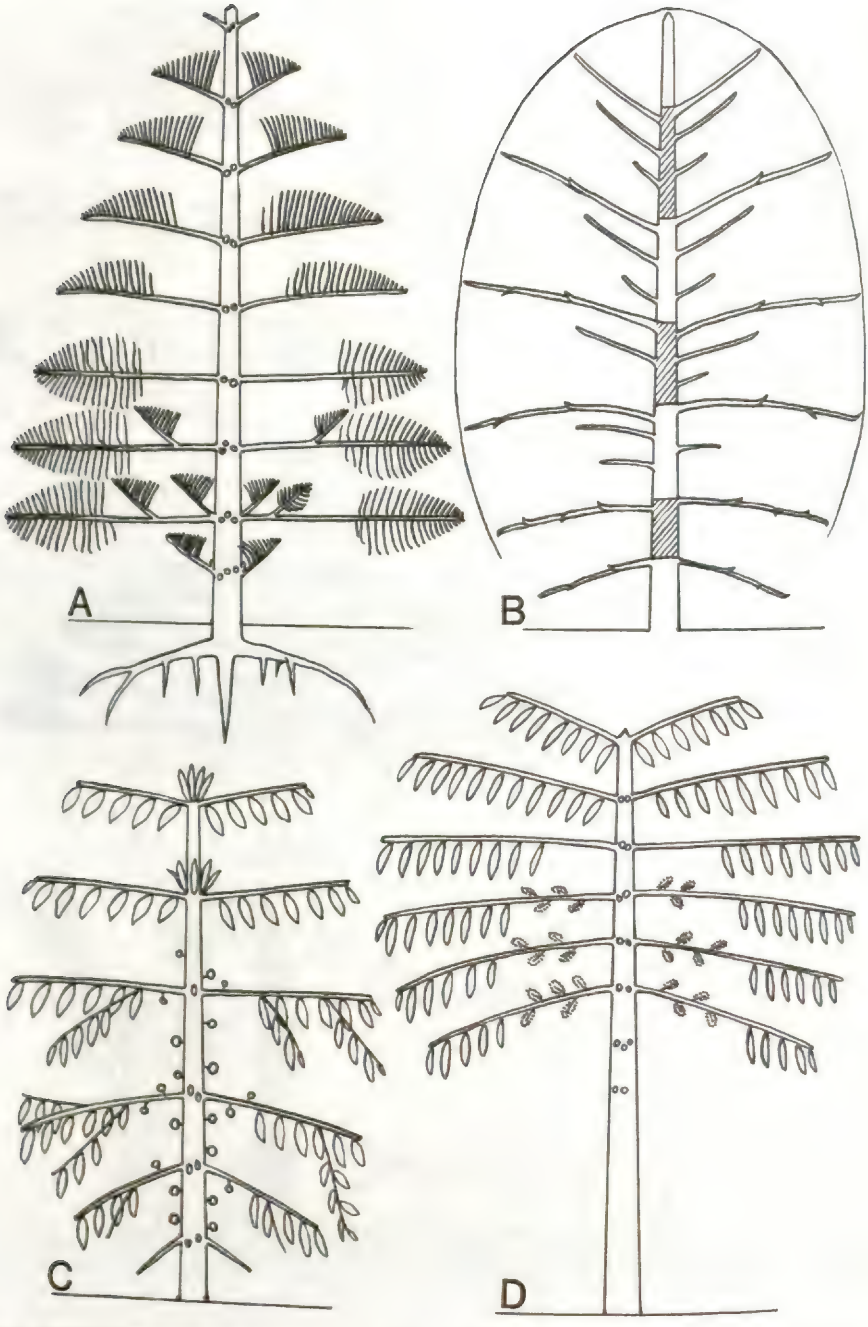


Figure 3. MASSART's model—four Architectural Units. A. (Araucariaceae) *Araucaria columnaris* (Fost.) Hooker: a young tree 6 m tall; the adult tree, which may reach 60 m, bears female cones, then male cones, at the top of the third order axes (= ramuli); from New Caledonia (Veillon, 1980). B. (Platanaceae) *Platanus hybrida* Brot.: a young tree 7 m tall with a characteristic sympodial structure; the adult tree, which reaches 30 m, bears apical flowers on high-order axes; from the Mediterranean region of Europe (Caraglio and Edelin, 1990). C. (Ebenaceae) *Diospyros matherana* A. C. Smith: a small, forest-understory tree up to 8 m tall, having monopodial axes and cauliflory; from French Guiana (Edelin and Hallé, 1985). D. (Myristicaceae) *Pycnanthus angolensis* (Welw.) Warb.: a tall tree of the African rain forest, up to 40 m; flowering is lateral (Hallé et al., 1978).

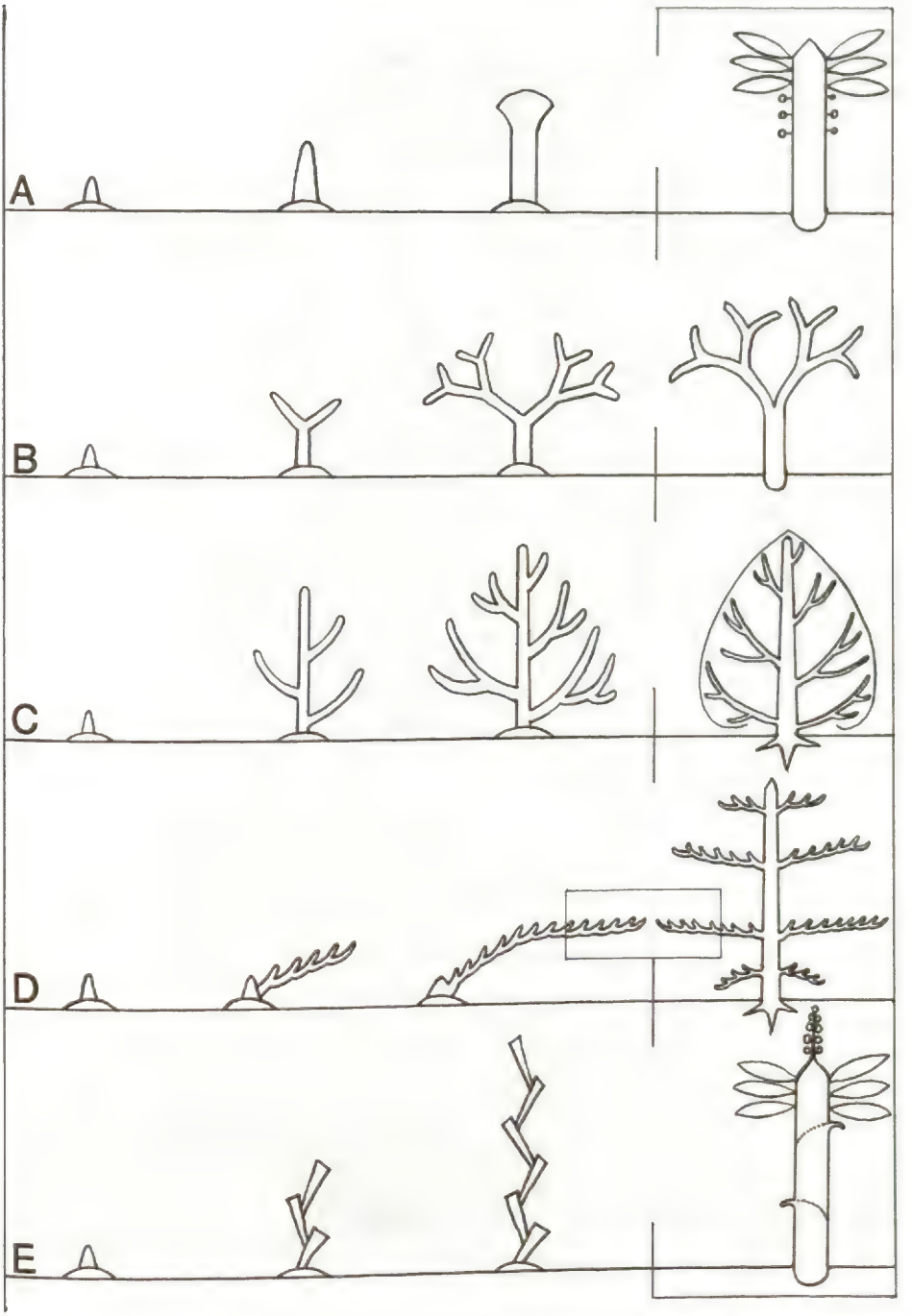


Figure 4. Architectural models adapted for corals, and their botanical equivalents (Dauget, 1991). A. CORNER's model—Examples: *Fungia fungites* L. or *Elaeis guineensis* Jacq. (Arecaceae). B. SCHOUTE's model—Examples: *Acropora palifera* Lmk. or *Hyphaene thebaïca* Mart. (Arecaceae). C. ATTIM's model—Examples: *Acropora cervicornis* Lmk. or *Rhizophora racemosa* Meyer (Rhizophoraceae). D. WOOD-JONES' model—Example: *Acropora hyacinthus* Dan. This form is equivalent to a single branch of AUBREVILLE's model (Example: *Terminalia catappa* L.). E. CHAMBERLAIN's model—Examples: *Lophelia pertusa* L. or male *Cycas circinalis* L.

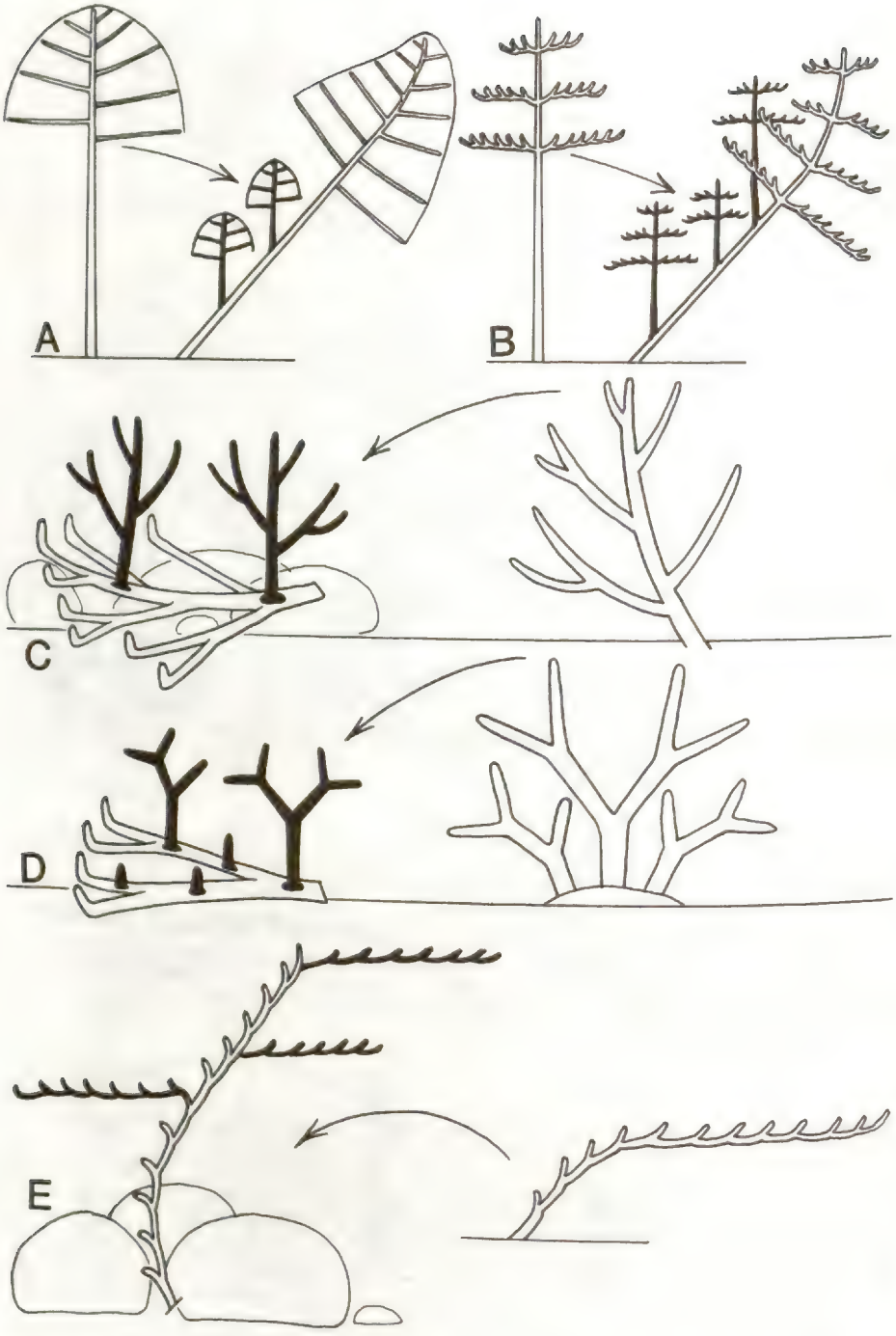


Figure 5. Reiteration in trees and corals (Dauget, 1985, 1991). A–B. Reiteration in trees: the development of previously dormant meristems on the upper surface of leaning trunks induces the growth of reiterated complexes (in black). The apex of the leaning tree continues to grow vertically. C–E. Reiteration in corals: the apices of the broken or leaning corals continue to grow vertically. Reiterated complexes in black. C. *Porites cylindrica* (SCHOUTE's model). D. *Acropora formosa* (ATTIM's model). E. *A. hyacinthus* (WOOD-JONES' model).

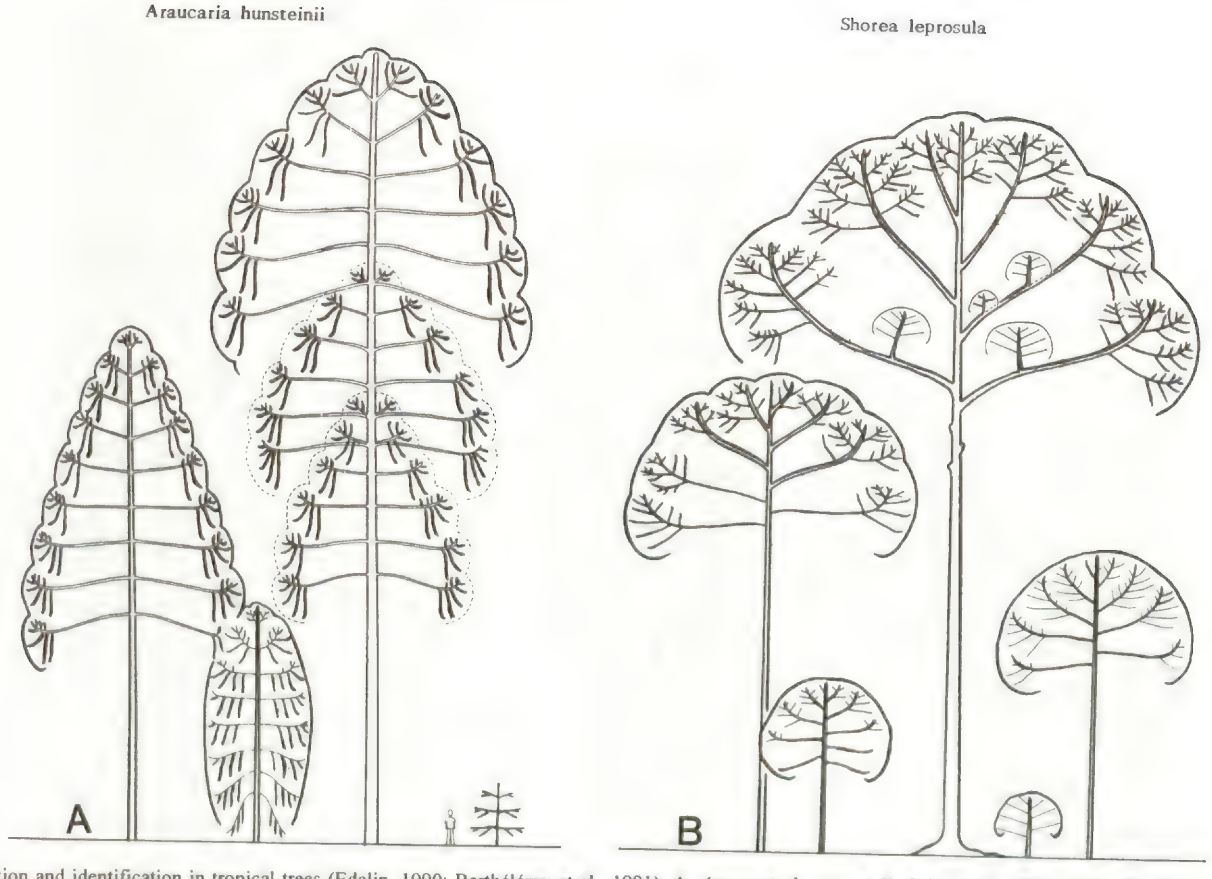


Figure 6. Reiteration and identification in tropical trees (Edelin, 1990; Barthélémy et al., 1991). A. *Araucaria hunsteinii* K. Schum., the "Klinkii Pine" of New Guinea. By means of a partial reiterative process, new branches are produced in the place of the old whorls, resulting in the development of "nesting crowns" that are produced in regular succession during the whole lifespan of the tree. B. *Shorea leprosula* Micq. This imposing tree in the Dipterocarpaceae is called "Meranti tembaga" in Indonesia. As it grows, the horizontal second order branches curve upwards and become huge reiterated complexes; on the most drooping of these limbs, some small reiterated complexes appear.

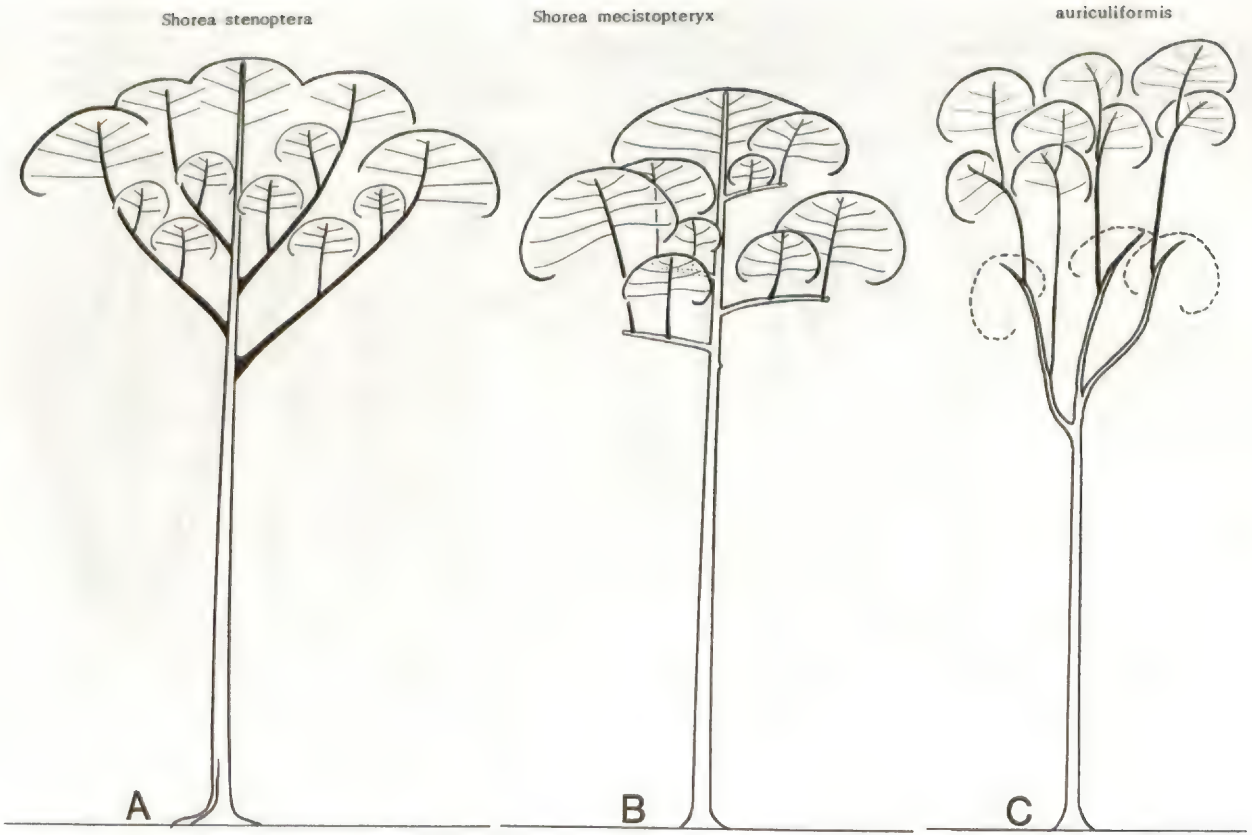


Figure 7. Reiteration and identification in tropical trees (Edelin, 1990; Barthélémy et al., 1991). A. *Shorea stenoptera* Burck is another Indonesian member of the Dipterocarpaceae. The reiterated complexes on the drooping limbs are much bigger than in *S. leprosula* (Figure 6B). B. *S. mecistopteryx* Ridl. On the upper surface of the drooping limbs, the reiterated complexes become so big that the apical part of the limb is pruned. This gives the crown a characteristic aspect. C. *Acacia auriculiformis* A. Cunn. ex Benth. (Mimosaceae): In this pioneer tree native of Northern Australia, the crown is constructed by the sympodial superposition of reiterated complexes. They behave like trees "grafted" on the top of one another. Here also, as in the four previous species, the tree is easily identified from a distance.

BRANCHING PATTERNS IN THE SOLANACEAE

Adrian D. Bell[1] and Trevor D. Dines[1]

ABSTRACT. Members of the Solanaceae exhibit a diversity of life forms, including trees, shrubs, lianas, and herbs. The branching architecture of the family has been shown to be both very precise and very diverse. This diversity appears to be non-correlative with the existing taxonomic framework, some genera being monotypic for architecture, others highly polytypic. A link may exist, however, between architecture and life form, and this possibility is examined from a functional rather than evolutionary viewpoint. The range of branching patterns so far described both support and extend existing architectural classifications. The precision of branching exhibited by many species results from well-defined patterns of bud potential. Once such patterns have been characterized, manipulation of bud potential is possible using reciprocal bud grafting techniques. The spatial and temporal constraints on bud determination can therefore be examined, and the ease with which intraspecific grafts can be made between genotypes allows for investigation of the hierarchical genetic control of bud determination.

Branching patterns within the Solanaceae are commonly very precise and predictable at the species level. However, descriptions of these patterns do not normally feature in taxonomic accounts of the family. Exceptions are to be found in Eichler (1875), Danert (1958, 1967), Child (1979), and Child and Lester (1991). In this paper we present a preliminary description of the range of branching patterns found, and relate these to recent advances in the field of plant architecture. Branching is a consequence of bud activity; the possibilities of assaying bud potential by surgical manipulation are described for the genus *Nicandra* Adans.

FLOWERING PLANT ARCHITECTURE

There has been considerable interest generated in recent years by the concept of plant architecture, based initially on the study of tropical trees (Hallé and Oldeman, 1970; Hallé, Oldeman, and Tomlinson, 1978; see also Hallé, this volume). A summary of refinements in this scheme is given in Bell (1991). In brief, it is recognized that the location, potential, and time of activity of buds in a young developing plant leads to the construction of a consistent pattern of branching for that particular species. The traditional format of a typical "Christmas tree" is a familiar example (*Picea abies* (L.) Karsten in Europe). The developmental sequences leading to a distinctive, indeed predictable, architecture such as this characterize what is referred to as an architectural model, each of which has been named after a botanist. The vast majority of woody plants so far studied can be allocated to one of just 23 models, listed in Appendix A. In some species the simple manifestation of a particular model is recognizable throughout the lifespan of the individual plant (e.g., *Picea abies*, model of Rauh; Edelin, 1977). For the majority of species, however, this first expression of the model, initiated by the epicotyl, is augmented progressively during the life of the individual by a process termed reiteration (see Hallé in this volume). The extent and timing of reiteration varies considerably between species (Edelin, 1990), but in its simplest expression one or more dormant buds will commence growth and initiate supplementary branching sequences, each being a complete replica of the original model. In time, a repetition of this process will result

[1]School of Biological Sciences, University of Wales, Bangor, Gwynedd, Wales, U.K.

in the development of an elaborate, and at first sight indecipherable, complex of branches. It is supposed that any plant species can be analyzed in terms of branching architecture, although the flexibility shown by individuals varies considerably in different taxa. Some are extremely conservative in this respect, e.g., the Solanaceae. Others are much more plastic (see Hutchings in this volume). Similarly, the range of different architectural models exhibited by different taxa varies enormously. Virtually all woody members of the family Fabaceae develop according to one architectural model (that of Troll), whereas Cremers (1976) found 10 different models amongst 12 species in the genus *Euphorbia* L. on Madagascar. The Solanaceae are similarly rich, with some 10 architectural models currently identified (see Figure 1 and Appendix A).

Although initially devised from observations of tropical trees, the architectural model concept can be applied to any growth form. Often the same developmental sequences and thus models are found to apply (model of Leeuwenberg amongst herbs, Mangenot amongst lianes, for example), but some architectures are found that have no counterparts in trees. This is to be expected and is not a problem; application of the concept to any plant group may reveal hitherto unappreciated features of form.

BRANCHING IN THE SOLANACEAE

The Solanaceae Juss. is a large subcosmopolitan family, organized into three subfamilies primarily on the basis of seed characteristics. These are the Solanoideae (with 1746 species in 60 genera), the Cestroideae (with 529 species in 34 genera), and the Nolanoideae (with 22 species in 2 genera) (D'Arcy, 1991). Members of the family vary in form from non-woody ephemerals to herbs, shrubs, lianes, and large forest trees. Some species develop unequivocally in accordance with one of the models of Hallé and Oldeman. Other species show a range of subtle variations on a theme, all of which could be classified under one model.

The product of growth of a single apical meristem is termed a module (Bell, 1991), and this can be either indeterminate (functioning indefinitely) or determinate (terminating in a flower, inflorescence, spine or tendril, for example). A sympodial axis is composed of a linear series of modules, each of which is usually determinate but can in some cases be indeterminate. All members of the Solanaceae are sympodial with determinate modules, with the possible exception of a very few entirely monopodial (i.e., indeterminate) species such as *Cestrum latifolium* Lam. and *Anthocercis viscosa* Haegi, which are yet to be confirmed.

Typically, the epicotyl axis (or primary module) always terminates in a flower after the production of a certain number of nodes (i.e., growth is determinate). All subsequent branches (and trunk if present) are built up from a series of modules each derived from an axillary bud and each again terminating in a flower. The exact disposition of these series of modules gives a distinctive range of architectures within the family that can be readily equated with tree models (Figure 1). Within the Solanaceae we have currently encountered 10 of the 23 architectural models described for flowering plants. These are, of course, limited to those models with determinate growth since all modules terminate with a flower (or inflorescence).

Solanaceae models can be classified following the original scheme of Hallé, Oldeman, and Tomlinson (1978) (Figure 1, see also Appendix A). Initially, the models are divided into monoaxial and polyaxial, the monoaxial being hapaxanthic species such as *Nicotiana tabacum* L. with a single determinate axis. Three types of polyaxial architecture can be identified: a) those with equivalent, undifferentiated axes not specialised into "trunk" or "branch" components (Chamberlain, Tomlinson, and Leeuwenberg), b) those with axes differentiated into distinct "trunk" and "branch" components (Koriba, Prevost, *Fremontoden-*

dron type), and c) those with mixed axes, i.e., each axis possessing an orthotropic (vertical) component and a plagiotropic (horizontal) component, the progressive superposition of axes leading to the construction of a trunk when present (Champagnat, Mangenot, Troll). Note that the "*Fremontodendron* type" is a category filling a gap in the original scheme; see Bell (1991) for further discussion. One of the most distinctive models is that of Prevost (polyaxial with differentiated axes, see Appendix A), represented by *Cyphomandra crassicaulis* (Ortega) Kuntze. In this species, two types of modules are present: orthotropic (vertical) trunk modules and plagiotropic (horizontal) branch modules. Each orthotropic trunk module terminates in an inflorescence, and plagiotropic branch modules form a subterminal tier of highly ramified branches just beneath it. This pattern of orthotropic trunk plus subterminal whorl of plagiotropic branches is repeated by proleptic (i.e., delayed) growth of a dormant bud on the last trunk module, just below the tier. The whole pattern is therefore constantly repeated, each "umbrella" on top of the last (Figure 1, Appendix A). This most precise architecture is fairly common, not only in Solanaceae but also in a number of unrelated families such as Apocynaceae and Icanthaceae.

Within Solanaceae it is not possible to find a clear-cut relationship between architectural formats and taxonomic groupings. Architecture is species-specific, but at the subfamilial and tribal levels there is considerable variation in model representation (Figures 2 and 3), with some taxa being monotypic and others polytypic. For example, the Salpiglossidae is a polytypic tribe with 79 species and five models, whereas the Lycieae is monotypic, with 82 species all conforming (according to current observations) to just one model, that of Champagnat. This may correlate with phylogeny, since the Salpiglossidae is polyphyletic (D'Arcy, 1991; Olmstead and Palmer, 1992) and the Lycieae is monophyletic (Bernardello, 1986). Similarly, at the generic level, the polytypic genus *Solanum* L. contains six models represented in over 1000 species, whilst the vast majority of the 95 species of *Nicotiana* L. develop according to the model of Holttum (except a few species; *N. glauca* Graham, which conforms to the model of Champagnat, and *N. alata* Link & Otto, conforming to the *Fremontodendron* type). Generally, some models are particularly common (such as Leeuwenberg and Champagnat), while others such as Koriba are rare.

The discrepancy between architectural model and taxonomy is not unexpected. Even at the generic level species exhibit a wide range of growth forms and to a certain extent this is reflected in the diversity of models present in the family. Nevertheless, the same model can be represented at all levels of stature—*Solanum mauritianum* Scop. is a 20 m tree whereas *Solanum luteum* L. with an identical sequence of branching is a 30 cm herb. Jeannoda-Robinson (1977) discusses the application of tree architecture to herbaceous plants and identifies three modes of expression: a) a simple reduction in size as indicated above; b) prostration, in which the branching format is the same as a tree model but the plant develops horizontally with its "trunk" growing along the ground (*Datura inoxia* Miller is an example); and c) fragmentation, in which the framework of the plant is recognizable as a portion of the whole model (for example, *Nicandra physaloides* (L.) Gaertner, in which only the first "trunk" and plagiotropic tier of the model of Prevost is expressed; see Figure 7).

HERBACEOUS ARCHITECTURES IN THE SOLANACEAE

Growth of solanaceous herbs is always sympodial. Successive modules terminate in a flower or cymose inflorescence and give rise to the next generation modules at a constant rate. Modules often bear just two leaf-like bracts (i.e., they are bifoliate), but may bear more (i.e., trifoliate for three or plurifoliate for more than three). The bud in the axil of a bract grows sylleptically to produce a daughter module. Its subtending bract, however, is often adnate to this daughter module, and is carried away from its original point of insertion on the

parent module to lie in a position beneath the terminal flower of the daughter module (Figure 4a, b). This process of adnation, termed recaulescence, is common in the Solanaceae (Child, 1979; Danert, 1958), along with concaulescence (in which part of the parent module itself is adnate to the daughter module), and obviously confuses the analysis of architecture. However, assuming that a module terminates in a flower (or cymose inflorescence) and bears two bracts, each with an axillary bud, two types of sympodial sequence can be identified:

a) Dichasial branching, where both axillary buds grow out at the same time and to the same extent, thus producing two daughter modules, each with a bract adnate to it (Figure 4b). Examples are *Solanum mauritianum*, *Brugmansia candida* Pers., and *Capsicum annuum* L.

b) Monochasial branching, where only one of the pair grow out, producing one relay module with adnate subtending bract. The other bud may remain dormant (Figure 4c) or grow out later (to a lesser extent), but its parent bract will not be adnate (Figure 4d). Examples are *Lycopersicum esculentum* Miller, *Hyoscyamnus niger* L., and *Physalis alkekengi* L.

Different species are consistent in the degree to which they incorporate the dichasial sequence, the monochasial sequence, or a combination of the two (such as *Capsicum annuum*, which reverts to monochasial branching after two generations of dichasial branching; see also Figure 6). At one extreme the main axis of the plant terminates in a single monochasium (e.g., *Physalis alkekengi*). At the other, the branching pattern is formed by constantly repeated dichasia. In reality, such a totally dichasial framework is rarely if ever encountered, but all possibilities between this and the single monochasium exist. Figure 5 shows diagrammatically this branching continuum where maximum bud activity (dichasial branching, 5A) would result in the model of Leeuwenberg and maximum control over bud growth (monochasial branching, 5E) results in the model of Chamberlain (compare Figure 1 and Appendix A).

Species from various genera can be allocated positions along the continuum depending on the degree of mono/di-chasiality. A critical factor is that a bud at the same relative position in the branching sequence in different species may be completely dormant, contribute to part of a monochasium, or form part of a dichasium. Any herb will thus lie somewhere on this continuum in accordance with its own particular pattern of bud determination.

A particularly common variant is that shown at the midpoint of the sequence, Figure 5C, where indefinite monochasial branching is preceded by two dichasial branches (e.g., *Capsicum annuum*). In solanaceous herbs generally, the first flower is followed by the production of one or two dichasial branches that then switch abruptly to monochasial branching, producing two or four identical axes. Preliminary observations suggest that this pattern (Figure 5C) is limited to members of the Solanoideae subfamily and is not encountered in the Cestroideae or the Nolanoideae. Each species will exhibit a particular pattern of branching—in *Lycianthes sanctaeclarae* (Greenman) D'Arcy (Figure 6), for example, the primary module is terminated by a flower and one dichasial branch, this producing two indefinite monochasia. The bud in the axil of the final leaf also produces a monochasium, and a tier of three plagiotropic branches is thus produced. A similar pattern is found in *Nicandra physaloides*, a more precise description of which follows.

BRANCHING IN *NICANDRA PHYSALOIDES*

Nicandra physaloides (tribe Nicandreae) is an annual herb from Peru, growing rapidly to 1–1.5m in greenhouse conditions. It germinates readily and is an ideal subject for experimentation.

The primary module (i.e., the epicotyl axis) is terminated by a single flower. This orthotropic axis bears 30–32 leaves in addition to the pair of cotyledons, each subtending a single bud. Two generations of dichasial branching are formed just beneath the terminal

flower, followed by monochasial sequences (Figure 5C). In addition to this, the bud in the axil of the most distal leaf on the primary module also produces a monochasial branch, ultimately resulting in a tier of five plagiotropic monochasia (Figure 7).

The buds along the primary module normally remain dormant but can usually be released from apical dominance by decapitation of the axis at the internode above. The number of nodes developing on the resulting shoot (a reiteration) before it forms its own terminal flower is a function of the bud's position on the primary module. The results of decapitation experiments (Dines, 1994) indicate that growth of proximal buds is generally accompanied by some neoformation, with more nodes on the reiterated shoot than in the dormant bud, while distal shoots express the same number of nodes on both dormant and reiterated shoots. Thus the fate of these latter buds is already determined at anthesis (which was the time at which decapitation took place) and their fate cannot be changed. Buds at more proximal positions have more flexible outcomes, developing extra internodes in response to decapitation in a position-dependent manner. They are, to an extent, undetermined, and thus are more competent to react to a determination signal. Similar gradients of bud potential on the primary module are known to occur in other species of Solanaceae such as *Datura stramonium* L., *Solanum nigrum* L. (Dines, 1994), and *Nicotiana tabacum* (McDaniel, Sangrey, and Singer, 1989).

If reiteration shoots formed in response to decapitation are allowed to continue development, a second gradient of potential is revealed (Figure 8). Distal buds (nodes 26–30) initiate monochasial axes in the manner of the distal bud that habitually develops as such in the intact plant. Proximal buds (below node 6) form a branching pattern identical to that of the whole plant in normal conditions. Intermediate buds form branch sequences on a gradient of complexity between these two extremes. Indeed, the gradient of potential along the main axis of *N. physaloides* is remarkably reminiscent of the continuum of patterns found along the taxonomic spectrum of the Solanaceae (Figure 5).

The relative ease with which bud grafts can be made in Solanaceae plants allows elucidation of the controls of determination, and perhaps, the genetic background to these controls. If, for example, a species-specific branching pattern is retained after grafting a bud from one species to another, this may indicate a closer alliance between these species than between ones in which the usual pattern is not expressed. Once patterns of determination have been characterized, as in *N. physaloides*, grafting buds of different ages to different positions (and even onto different species) will aid identification of the controls of bud determination, and measure the variability of these controls across the family.

CONCLUSIONS

The Solanaceae is a large and diverse family, including a wide variety of life forms and branching architectures. The original synthesis of tree architecture by Hallé, Oldeman, and Tomlinson (1979) includes concepts that are directly applicable to both woody and herbaceous species, and simplifies the analysis of the complex architectures encountered. The highly modular growth exhibited by almost all species limits the number of models to those with determinate growth, but it may be that the evolution of monopodiality is an independent development in a very few, unrelated taxa. The reduction of the inflorescence is one evolutionary trait identified by Child (1979), but no such sequence is clearly observed in a consideration of whole plant architecture—different evolutionary paths may produce strikingly similar architectures, and continuums of branching patterns must not be regarded as phylogenetic sequences but simply as a range of potentials expressed between two extremes. Only where a detailed phylogenetic history is available, based on other characteristics, could the development of an architecture be followed.

A constant source of speculation is how unrelated families produce identical architectures; for example, Prevost also occurs in Boraginaceae and Apocynaceae, Champagnat in Rosaceae and Sterculiaceae, and Leeuwenberg in Euphorbiaceae, Liliaceae, and Araliaceae. The degree of similarity between some growth forms and adaptions implies a high degree of convergent evolution, but the reason why so few models (23) are identified amongst so many different species is unclear. Similarly, within a single genus, the range of architectures expressed can be so variable that each growth form may be conceived as being subject to very different selective pressures. In *Nicotiana*, for example, the monocarpic condition is retained, leading to the predominant expression of the model of Holttum. The three species of *Leptoglossis* Benth., however, all exhibit different models; *L. lomana* (Diels) Hunziker is a diminutive ephemeral (model of Holttum), *L. linifolia* (Miers) Griseb. is a small perennial (model of Tomlinson), while *L. schwenkioides* Benth. is a large shrub (model of Champagnat). This diversity is to be expected, however, when the model is correlated not with taxonomy but with life form. In the above example, the only real difference between *L. lomana* and *L. linifolia* is the development of perennation in the latter—repetition of identical modules leads to the identification of another model. Not enough is presently known about the relationship between phylogeny and architecture to make any generalizations, and it is probable that each scenario will be more or less original for that particular group. We are presently examining the American genus *Jaltomata* Schldl. for evidence of architectural evolution, since the phylogeny of the group has been evaluated (Mione, pers. comm.), and it appears that woody species may have evolved from herbaceous ones. On a broader scale, it is possible that one pattern of branching (Figure 5C) is limited to the Solanoideae subfamily, where it forms a basic architectural unit intermediate between the "module" and the "model." It is constantly expressed during the construction of herbaceous and woody species, and has no known counterpart in either of the other subfamilies. Since the Cestroideae are considered ancestral, it may be that this particular architecture arose during evolution of the Solanoideae and is unique to it. It is hoped that further observations will clarify the evolution of branching in the Solanaceae (Dines, 1994).

That the same controls over branching architecture exist between most species of the family is unequivocal. Particularly in herbaceous species, the determination of apical meristems takes place early in the sexual phase—development in one or more axillary meristems leads to the potential for either monochasial or dichasial branching well before expansion of the axis. The similarity in basic architecture between so many species suggests that one pattern is ancestral to the whole group: the production of four indefinite monochasia after two generations of dichasial branching. This pattern is modified, however, to produce a range of architectures; for example, lateral branches may or may not develop, the primary module may be prostrated or remain erect, or further repetitions of the unit may be produced. The stability of these characteristics, and the control of their expression, may be examined in a variety of ways. Decapitation experiments can be used to identify patterns of bud potential that result from temporal and spatial determination signals, which are probably hormonal. Novel bud-grafting techniques (such as switching buds from one location to another and from one species to another) can then be implemented to investigate both the flexibility of determination within a species, and the degree to which determination signals are species-specific; can one species adopt the inflorescence branching pattern of another, and if so, are some branching patterns common to many species while some are highly characteristic of a few? It is clear that the typical herb constitutes two very different populations of buds (those of the primary module and those of the inflorescence) and that these populations behave differently as regards patterns of determination. How different these patterns are between species remains to be evaluated.

LITERATURE CITED

Bell, A. D. 1991. Plant Form. Oxford University Press, Oxford.

Bernardello, L. M. 1986. Revisión taxonómica de las especies sudaméricas de *Lycium* (Solanaceae). Bol. Acad. Nac. Ci. 57: 174–356.

Child, A. 1979. A review of branching patterns in the Solanaceae. Pp. 345–356 *in* J. G. Hawkes, R. N. Lester, and A. D. Skelding (eds.), The Biology and Taxonomy of the Solanaceae. Academic Press, London.

Child, A. and R. N. Lester. 1991. Life form and branching within the Solanaceae. Pp. 151–159 *in* J. G. Hawkes and R. N. Lester (eds.), Solanaceae III: Taxonomy, Chemistry, Evolution. Royal Botanic Gardens, Kew, London.

Cremers, G. 1976. Sur la présence de dix modèles d'architecture végétative chez les Euphorbes malgaches. Compt. Rend. Hebd. Séances Acad. Sci., Sér. D 281: 1575–1578.

Danert, S. 1958. Die Verzweigung der Solanaceen in reproduktiven Bereich. Abh. Deutsch. Akad. Wiss. Berlin, Kl. Chem. 6: 1–183.

Danert, S. 1967. Die Verzweigung als infragenerisches Gruppenmerkmal in der Gattung *Solanum* L. Kulturpflanze 15: 275–292.

D'Arcy, W. G. 1991. The Solanaceae since 1976, with a review of its biogeography. Pp. 75–137 *in* J. G. Hawkes and R. N. Lester (eds.), Solanaceae III: Taxonomy, Chemistry, Evolution. Royal Botanic Gardens Kew, London.

Dines, T. D. 1994. The branching architecture of the Solanaceae and the role of bud potential and determination. Ph. D. Thesis. University of Wales, Bangor, U. K.

Edelin, C. 1977. Images de l'architecture des conifères. Thèse, Docteur de Specialité de Sciences Biologiques. Université des Sciences et Techniques du Languedoc, Montpellier, France.

Edelin, C. 1990. The monopodial architecture: The case of some tree species from Tropical Asia. FRIM Research Pamphlet No. 105, Forest Research Institute Malaysia Publication.

Eichler, A. W. 1875. Blüthendiagramme, I. Leipzig.

Hallé, F. and R. A. A. Oldeman. 1970. Essai sur l'architecture et la dynamique de croissance des arbres tropicaux. Collection de monographies de Botanique et de Biologie Végétale, 6. Masson et Cie, Paris.

Hallé, F., R. A. A. Oldeman, and P. B. Tomlinson. 1978. Tropical Trees and Forests: An Architectural Analysis. Springer, Berlin.

Jeannoda-Robinson, V. 1977. Contribution à l'étude de l'architecture des herbes. Thèse, Docteur de Specialité de Sciences Biologiques. Université des Sciences et Techniques du Languedoc, Montpellier, France.

McDaniel, C. N., K. A. Sangrey, and S. R. Singer. 1989. Node counting in axillary buds of *Nicotiana tabacum* cv. Wisconsin 38, a day-neutral plant. Amer. J. Bot. 76: 403–408.

Olmstead, R. G. and J. D. Palmer. 1992. A chloroplast DNA phylogeny of the Solanaceae: subfamilial relationships and character evolution. Ann. Missouri Bot. Gard. 79: 346–360.

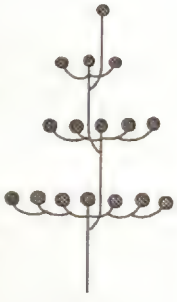
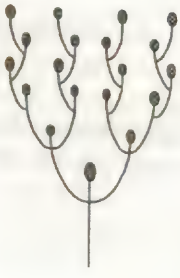
Monoaxial	Polyaxial		
	Axes Equivalent	Axes Specialized	Axes Mixed
HOLTTUM	CHAMBERLAIN	KORIBA	CHAMPAGNAT
	TOMLINSON	PREVOST 	MANGENOT
	LEEUWENBERG 	F'DENDRON TYPE	TROLL

Figure 1. Classification of the architectural models found in the Solanaceae.

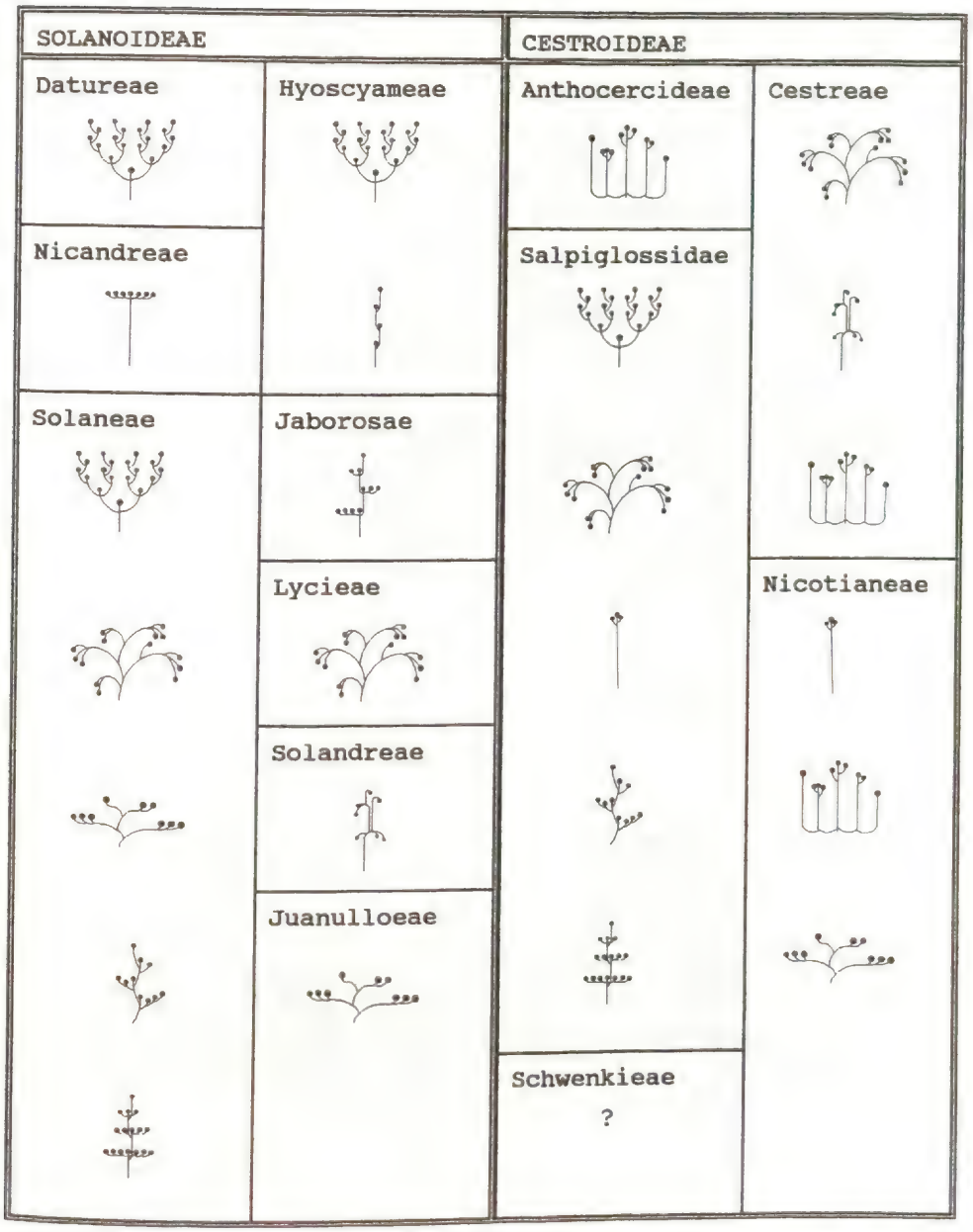


Figure 2. Distribution of architectural models in tribes of the Solanoideae and Cestroideae subfamilies. The model of Tomlinson is the only one observed to date in the subfamily Nolanoideae (not shown). Refer to Figure 1 for architectural symbols.

SOLANOIDEAE 1746 species	CESTROIDEAE 529 species	NOLANOIDEAE 22 species
Leeuwenberg Champagnat Troll Mangenot Prevost F'dendron Type Tomlinson Chamberlain Koriba	Leeuwenberg Champagnat Troll Mangenot Prevost F'dendron Type Tomlinson Holttum	Tomlinson
9 MODELS	8 MODELS	1 MODEL

Figure 3. Distribution of architectural models within subfamilies of the Solanaceae.

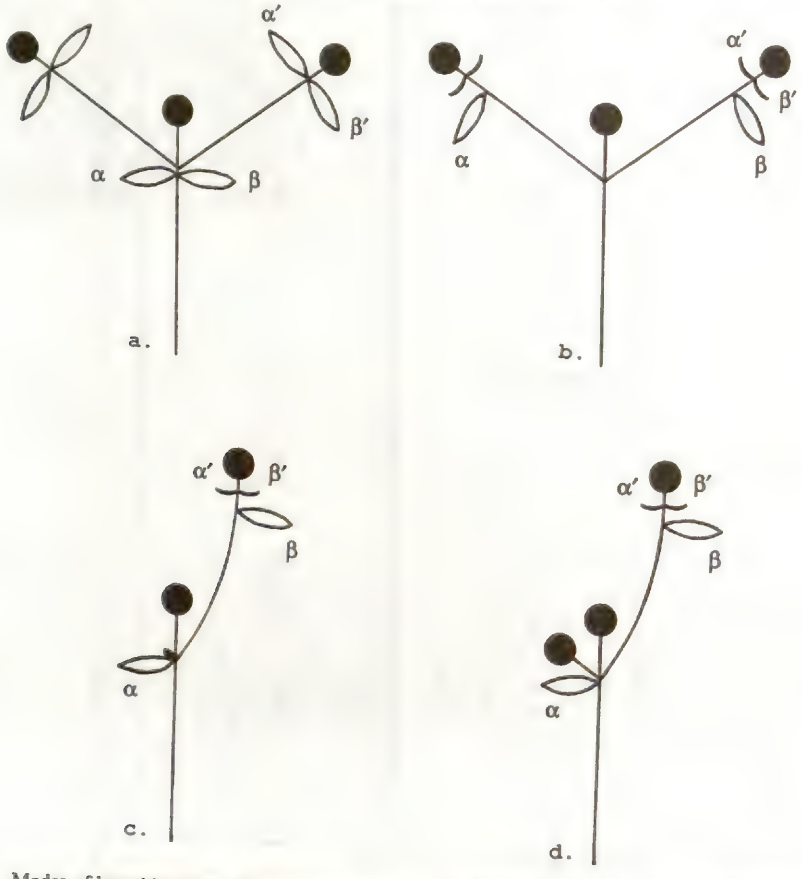


Figure 4. Modes of branching in the Solanaceae. a) Typical solanaceous dichasium, b) solanaceous dichasium, bracts adnate, c) solanaceous monochasium with dormant bud and one adnate bract, d) solanaceous monochasium with active bud, one adnate bract and one sessile bract. See text for further explanation.

Figure 5. Continuum of Solanaceae architectures, from fully dichasial (A) to fully monochasial (E) branching. Various species can be arranged along this continuum as illustrated.
NB. Monochasial and dichasial components are transposed into two dimensions for convenience of illustration.

DICHASIAL

MONOCHASIAL

A	B	C	D	E
Not found	Bruqmansia candida	Capsicum annuum	Lycopersicon esculentum	Hyoscyamnus niqer
	Datura sanquineum	Datura stramonium	Solanum sisymbriifolium	Jaltomata edulis
	Dunalia lorentzii	Solanum luteum	Solanum robustum	Physalis alkekenqi
	Solanum mauritanum	Saracha umbellata	Salpichroa origanifolia	Solanum aubletii

Figure 6. Top: Photograph of *Lycianthes sanctaeclarae* (Solanoideae). Bottom: Branching architecture of *L. sanctaeclarae*. The orthotropic module (O) is terminated by an aborted flower (T). A dichasial branch then produces two monochasia (M). The axillary bud of the last leaf before the terminal flower has produced a third monochasium (M_1). This architecture is a variation of Figure 5C, and is thus one point in a whole continuum of branching patterns.

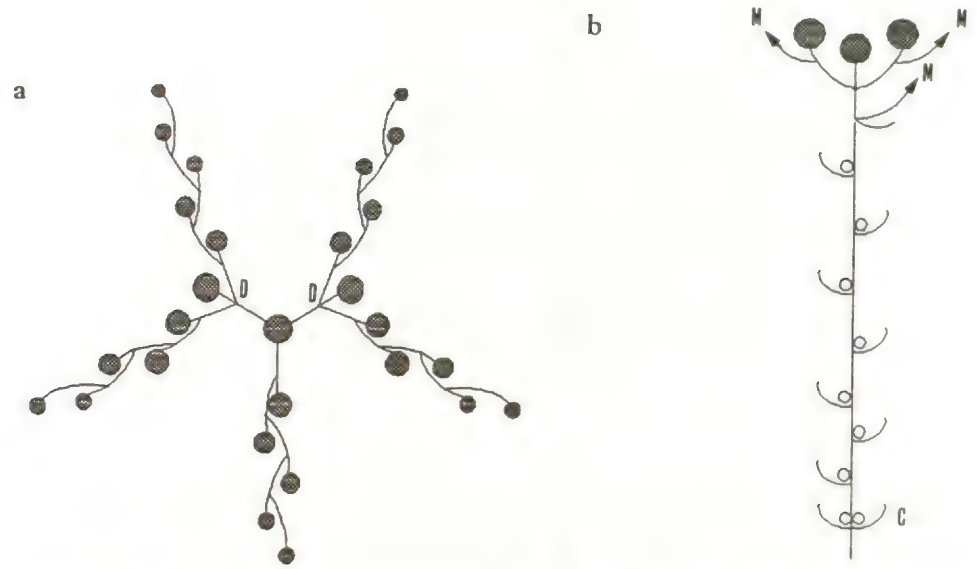


Figure 7. Branching architecture of *Nicandra physaloides*. a) plan view, b) vertical view (with monochasia reduced for clarity). D - dichasia, M - monochasia, C - cotyledons.

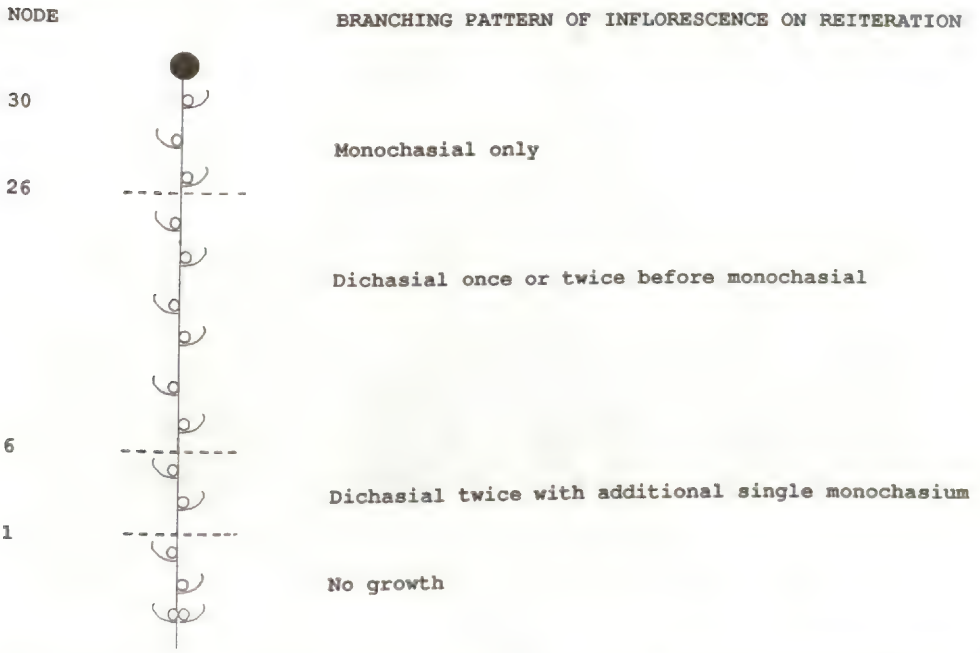
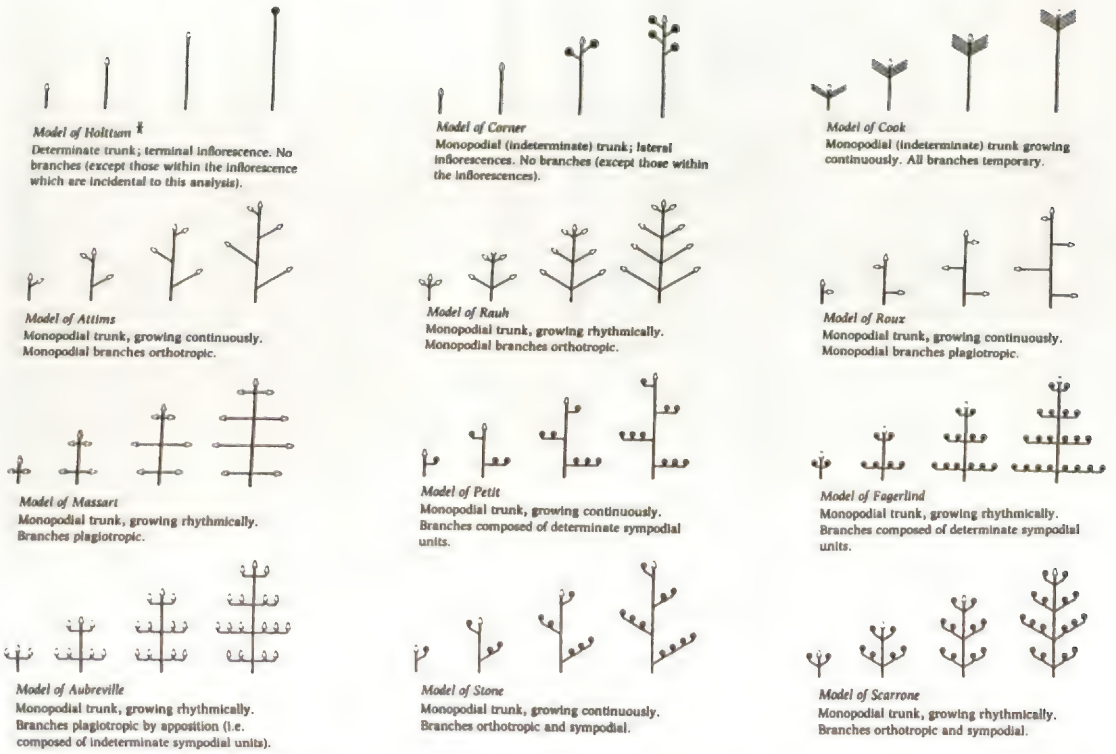


Figure 8. Branching patterns of inflorescences on reiterations growing from different levels of the primary module of *Nicandra physaloides*. This illustrates that inflorescence architecture is position dependent, with the complete model (Figure 7) being expressed only by growth of the lowest buds.


Model of Holttum *
Determinate trunk; terminal inflorescence. No branches (except those within the inflorescence which are incidental to this analysis).
Model of Attims
Monopodial trunk, growing continuously. Monopodial branches orthotropic.
Model of Massart
Monopodial trunk, growing rhythmically. Branches plagiotropic.
Model of Aubreville
Monopodial trunk, growing rhythmically. Branches plagiotropic by apposition (i.e. composed of indeterminate sympodial units).
Model of Corner
Monopodial (indeterminate) trunk; lateral inflorescences. No branches (except those within the inflorescences).
Model of Rauh
Monopodial trunk, growing rhythmically. Monopodial branches orthotropic.
Model of Petit
Monopodial trunk, growing continuously. Branches composed of determinate sympodial units.
Model of Stone
Monopodial trunk, growing continuously. Branches orthotropic and sympodial.
Model of Cook
Monopodial (indeterminate) trunk growing continuously. All branches temporary.
Model of Roux
Monopodial trunk, growing continuously. Monopodial branches plagiotropic.
Model of Fagerlind
Monopodial trunk, growing rhythmically. Branches composed of determinate sympodial units.
Model of Scarrone
Monopodial trunk, growing rhythmically. Branches orthotropic and sympodial.

Appendix A. A summary of the 23 architectural models of Hallé et al. (1978). Each model is represented by a developmental sequence. Black dots indicate flowering; open arrowheads indicate continued growth of an apical meristem. Compiled and compressed from Bell (1991).

* Indicates a model encountered in the Solanaceae.

Model of Troll ‡

The trunk and branches are plagiotropic except perhaps for a short proximal portion. The monopodial trunk is secondarily reorientated into the vertical position by cambial activity.

Model of Mangenot ‡

Orthotropic sympodial trunk, the distal portion of each sympodial unit developing sideways as a plagiotropic branch.

Model of Champagnat ‡

Orthotropic sympodial trunk, the distal portion of each sympodial unit developing sideways and drooping under its own weight.

Model of McClure

Sympodial branching sequence in which the proximal part of each determinate sympodial unit is plagiotropic and the distal part forms an orthotropic trunk. The trunk bears determinate branches.

Model of Tomlinson ‡

Sympodial branching sequence with each orthotropic sympodial unit borne on the proximal portion of a previous unit. Sympodial units indeterminate or determinate.

Model of Chamberlain ‡

Sympodial trunk, no branches. Each sympodial unit bears just one similar unit at its distal end.

Model of Leeuwenberg ‡

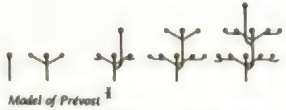
Sympodial branching sequence. Each sympodial unit bears more than one similar unit at its distal end.

Model of Schoute

True dichotomy of the apex at intervals. Flowering lateral.

Model of Koriba ‡

Sympodial trunk. Each sympodial unit of the trunk bears more than one laterally extending branch at its distal end. One of these branches is secondarily reorientated into the vertical position to become the next trunk unit



Model of Prévost ‡

Sympodial trunk. Each sympodial unit of the trunk bears more than one branch at its distal end. One of these branches is delayed in its extension and grows vertically to become the next unit of the trunk. The other branches are initially orthotropic but become plagiotropic by apposition or substitution

Model of Nozeran ‡

Sympodial trunk. Each sympodial unit of the trunk bears more than one branch at its distal end. One of these branches is delayed in its extension and grows vertically to become the next unit of the trunk. The other branches are plagiotropic, retaining this character even if rooted as cuttings

Appendix A. Continued.

PLASTICITY OF BRANCHING PATTERNS IN THE CLONAL HERBS *TRIFOLIUM REPENS* L. AND *GLECHOMA HEDERACEA* L.

Michael J. Hutchings[1] and Roy Turkington[2]

ABSTRACT. Phyllotaxy—the spiral arrangement of leaves on a stem—is a stable and therefore reliable taxonomic character. It is under strong genetic control and unaffected by environmental conditions. In contrast, the growth of axillary buds from the leaves of a plant to produce structural branches is unreliable as a taxonomic character for at least two reasons. First, ontogenetic changes in growth rules alter the probability of growth of individual buds. Secondly, apical control of lateral bud development differs markedly as biotic and abiotic growing conditions change and as carbohydrate supply alters. Results are presented illustrating and interpreting variation in branching both between plants and within different parts of the same plant growing under a range of abiotic and biotic conditions. Particular attention is paid to the significance of flexibility in branching patterns in the clonal herbs *Trifolium repens* and *Glechoma hederacea*.

INTRODUCTION

The types of morphological characteristics of plants that are of interest to taxonomists and ecologists tend to rest on either side of a dichotomy. Taxonomists are interested in characteristics that are stable, but ecologists find such characteristics uninteresting because they do not respond to experimental treatments. However, ecologists want reassurance from taxonomists that stable, reliable characteristics can be recognized easily, enabling ready identification of species under investigation.

Phyllotaxy—the spiral arrangement of leaves on a stem—is a good example of a stable plant characteristic. It is strongly controlled by genotype and is almost unresponsive to environmental conditions (Hardwick, 1986). For example, Meicenheimer (1981, in Hardwick, 1986) found a standard error of only 0.26° in the angle between successive elements in the phyllotactic spiral of *Epilobium*. This standard error in position of leaf insertion corresponds to a distance of only approximately one cell diameter on the stem surface. Whereas the positions of insertion of leaves upon a stem are closely controlled, however, other characteristics associated with leaves, such as the growth of buds from leaf axils to produce structural branches, are much less stable. The first purpose of this paper is to describe the variability in this characteristic in the clonal species *Trifolium repens* and *Glechoma hederacea*, in response to different growing conditions.

Several studies involving *T. repens*, *G. hederacea*, and other species with stoloniferous and rhizomatous growth forms have shown that they respond to local growing conditions through morphological plasticity and that one of the most plastic characteristics is the growth and development of branches from axillary buds. As modular plants grow, branching at their nodes causes the density of their apices to increase; thus, as the dimensions of the plant show a linear increase, the number of growing points will increase exponentially, unless growth rules alter so that more buds remain dormant (Hardwick, 1986). To compensate, there must be either an ontogenetic reduction in the probability of a bud growing as the plant expands, or a decline in this probability caused by the increasing local density of growing points. However, despite any such programmed change, recent studies have shown that the

[1]School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex, BN1 9QG, U.K.
[2]Department of Botany, University of British Columbia, Vancouver, B. C., CANADA V6T 1Z4.

behavior and fate of individual buds are often affected more by their local environment than by their position in the ontogeny of the plant (e.g., Franco, 1986; Jones and Harper, 1987a,b); the examples described below have been chosen to illustrate this for clonal herbs.

While the fate of buds is strongly dependent upon local conditions, it is now apparent that clonal species can show either a whole-clone integrated response to the variety of growing conditions that are experienced, or localized responses, in which different sub-structural units of the clone react quasi-independently to specific local growing conditions. Those clonal species that display localized responses to growing conditions have structures that are partitioned into a number of integrated physiological units (IPUs; Watson and Casper, 1984). IPUs are structural elements within which assimilation, distribution, and utilization of carbon, and perhaps other substances, is regulated. Although there is some dispute about the extent of integration between parts of *T. repens* clones (Harvey, 1970; Hoshino, 1974; Newton, 1986; Chapman, Robson, and Snaydon, 1991, 1992), it is clear that this species is more functionally integrated than *G. hederacea*, in which partitioning of structural branches into IPUs has been shown to be a consequence of the anatomy of the vascular system (Price, Marshall, and Hutchings, 1992; Price and Hutchings, 1992). The second purpose of this paper is to examine the consequences for the morphological development of clonal species of having either widespread physiological integration or IPUs, in a heterogeneous environment. Frequency of branching is again used as a major variable with which to illustrate the text.

THE SPECIES

Clonal species that possess stolons and rhizomes are able to spread laterally. Morphological plasticity confers upon at least some of these species a capacity to alter the extent to which they explore and exploit their habitat in response to local habitat quality (Slade and Hutchings, 1987a–c). The capacity for lateral movement is highly developed in *T. repens* and *G. hederacea*, both of which produce horizontally-oriented stems. Such stems are conveniently referred to as stolons. In heterogeneous habitats the stolons grow through microenvironments that have a variety of abiotic conditions and neighbor species. As they do so they leave a succession of modules or ramets (feeding sites, at which photosynthesis and/or uptake of nutrients and water can take place) experiencing different conditions (Bell, 1984). Thus adjacent modules or ramets (the latter term is used throughout the remainder of this paper) on a single stolon, or on different stolons, may explore patches of habitat with differing biotic and abiotic properties.

The ramets produced by both *T. repens* and *G. hederacea* have determinate forms. *Trifolium repens* (Leguminosae) is often the dominant dicotyledonous species in permanent grassland in temperate areas of the world, growing with a number of grass species and with other dicots. Its stolons can exceed 1m in length and bear more than 50 ramets, each consisting of a node and associated internode, a trifoliate leaf lamina, a petiole, and an axillary bud. When all axillary buds develop, horizontal branches are produced alternately on either side of the parent stolon (Figure 1a).

Glechoma hederacea (Lamiaceae) grows in permanent grassland and in moderately shaded habitats. Its stolons can be over 1m in length, and can bear several nodes at intervals of up to 12cm. At each node the plant produces a ramet consisting of two leaves each on a vertical petiole (Figure 1b). The stolons are square in cross section, and the two leaves at a given node emerge from opposite sides of the stolon. Leaves at successive nodes are set at right angles to each other, giving the opposite decussate phyllotaxy characteristic of the family Lamiaceae. Thus the leaves are set in four ranks or orthostichies (Figure 1c). Buds in the axils of each leaf give *G. hederacea* the capacity to produce two stolon branches at each node.

MORPHOLOGICAL RESPONSES OF *TRIFOLIUM REPENS* TO NEIGHBORS

In an experiment by Turkington, Sackville Hamilton, and Gliddon (1991), individual clones of *Trifolium repens* were grown so that all of the ramets on one side of the main stolon were growing with one species of grass while on the other side of the same *T. repens* they were growing with a different species of grass. The size of individual ramets developed with one species of grass was different from the size of ramets developed on the other side, but it was not dependent upon the species of grass on the other side of the same plant. This result is consistent with an hypothesis of a local response to neighbors, whereby ramets respond solely to their immediate environment. In contrast, while the number of ramets was the same on both sides of the plant, it differed on plants growing with different pairs of grass neighbors. These differences were determined primarily by the rate at which individual meristems produced new ramets. This is consistent with an hypothesis of a global response, in which all apices of a single plant are integrated so that they produce ramets at the same rate, even if they occupy different microenvironments. This suggests that no single architecture is universally advantageous. Averaged over all environments tested, the different architectures are simply different ways of achieving the same dry weight, but different architectures are specifically advantageous in different environments.

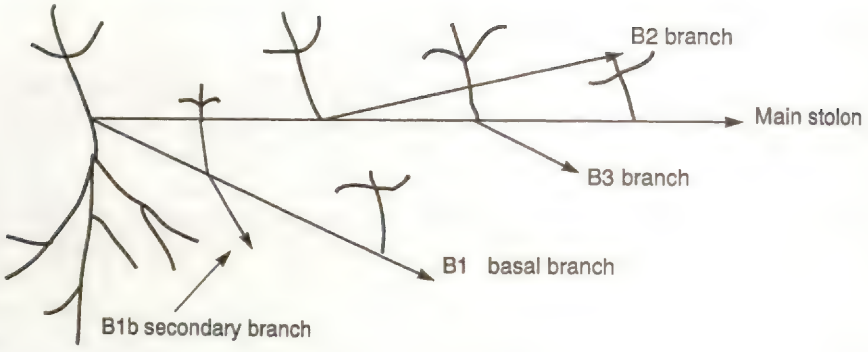
A possible physiological explanation for these results is that whereas the development of individual ramets is dependent mainly upon resources acquired by the ramet itself (Harvey, 1979; Newton, 1986), the resources used by an apex in producing new ramets are obtained from several other connected ramets (Hoshino, 1975; Harvey, 1979). A possible ecological explanation argues that the growth habit of *T. repens* enables it to explore a heterogeneous environment, exploiting locally favorable environments by branching profusely, and passing through locally unfavorable environments by extending the main stolon axis without branching. This hypothesis requires that a branch be initiated in response to the local microenvironment, whereas production of new ramets by an apex should continue regardless of the local microenvironment of the apex, exactly as shown by the results of Turkington, Sackville Hamilton, and Gliddon (1991).

The various responses described for *T. repens* are presumably not directly due to the physical effects of neighbors on the clover. Rather, it is assumed that neighbors alter local microenvironments in subtle ways that in turn elicit a growth response in *T. repens*. Some of these indirect effects are considered in the following sections.

MORPHOLOGICAL RESPONSES OF *TRIFOLIUM REPENS* TO LIGHT INTENSITY AND LIGHT QUALITY

Low levels of photosynthetically active radiation (PAR) have long been known to enhance apical dominance and promote internode extension. A similar effect in clonal species with horizontally-growing stems would reduce branching and favor unidirectional growth; this is known to happen in some species with such a growth form, including *Glechoma hederacea* (Slade and Hutchings, 1987b). A low ratio of red/far red (R/FR) light incident on the plant also produces these responses. Such a R/FR ratio is found in light that has been transmitted through a canopy of leaves. One benefit of these phenotypic responses is that in a heterogeneous habitat the clone can quickly vacate sites with low light intensity or altered light quality, and raise the probability of encountering more favorable light conditions.

An illustration of the phenomenon is provided by an experiment by Solangaarachchi and Harper (1987), in which *T. repens* clones were grown either in control conditions with no



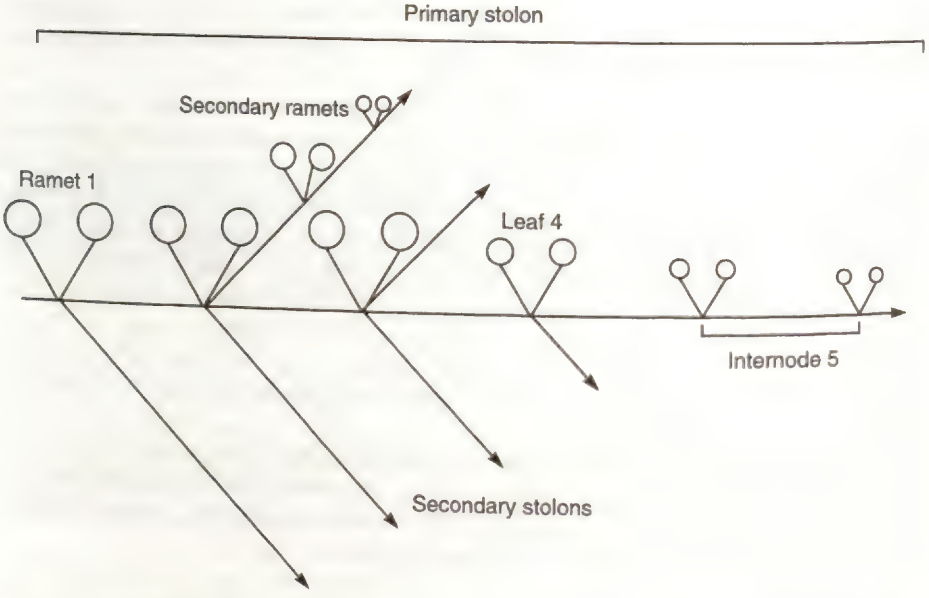


Figure 1. Diagrammatic representation of the pattern of vegetative growth in (a, top) *Trifolium repens* (after Kemball, Palmer, and Marshall 1992), and (b, bottom) *Glechoma hederacea*. c, facing page: A diagrammatic cross section through a primary stolon of *Glechoma hederacea*, showing the relationship between the four vascular bundles (circles) in the primary stolon, and the leaf traces of ramets at node N (arrows with white heads) and at node N+1 (arrows with black heads). Each of the four vascular bundles in the stolon is illustrated with a different type of shading. The same shading extends into the leaf traces to show the vascular continuity between the stolon and the leaves.

shade, under broken canopies of black polythene "leaves" providing neutral shade, or under broken canopies of *T. repens* leaves that provided shade with an altered spectral quality. The density of leaves in both types of canopies was manipulated so that the test clones received 50% and 30% of control PAR. The R/FR ratio did not differ significantly between the polythene shade treatments and the control, but it was significantly lower in the *T. repens* canopy treatments. There was also a significant fall in R/FR ratio from the *T. repens* canopy treatment providing 50% PAR to that providing 30% PAR.

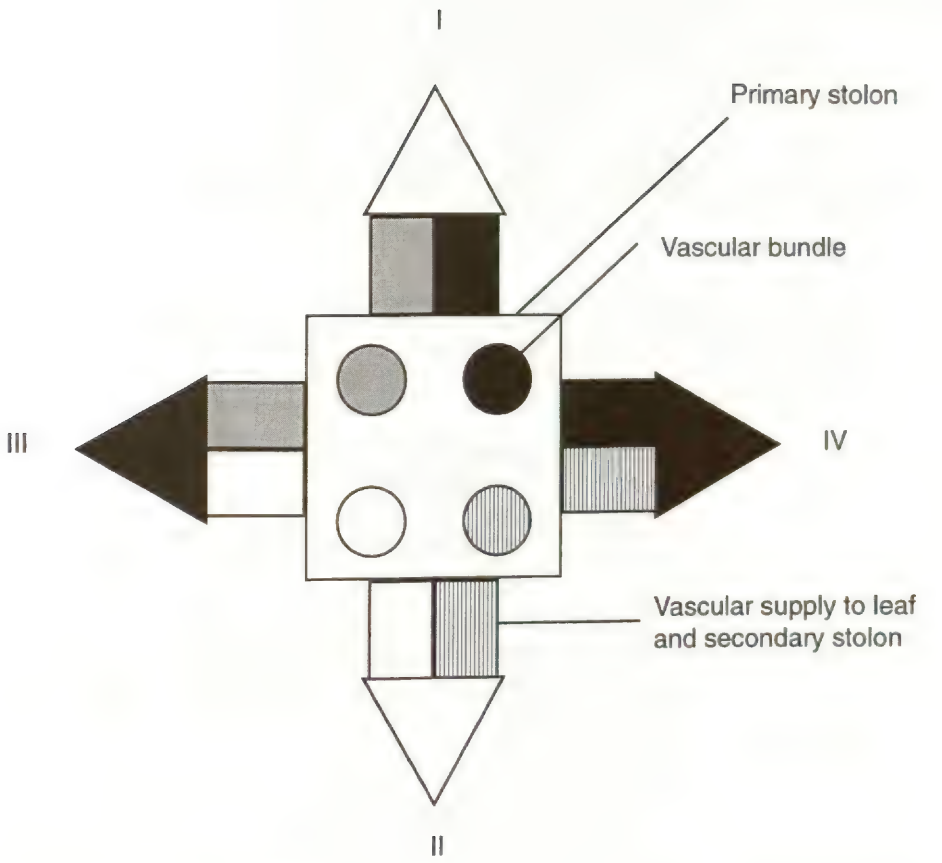


Figure 1c. See caption on facing page.

The most relevant results in the context of the present discussion concern the number of nodes on primary stolons, and the proportion of these nodes that branched on clones in the different shading treatments. Shading had a moderate effect upon node production on primary stolons, but branching frequency at these nodes was substantially lower in all shade treatments than in the control clones, and declined further with reductions in PAR (Table 1). Moreover, for a given level of PAR, the frequency of branching was lower when clones were shaded by *T. repens* canopies (Table 1). Thus, this experiment shows that the quantity and quality of shade cast on *T. repens* has a marked influence on its branching frequency. The sharp reduction in branching frequency at the primary stolon nodes resulted in a very marked fall in total node production by the whole clone. At a given PAR level, total node production was decreased to a greater extent in light with a reduced R/FR ratio.

More detailed analysis of this phenomenon demonstrates that the extent of branching suppression displayed by *T. repens* under shading by competitors is species-specific, and that a major cause of this is differences in the exact spectral quality of shade light transmitted by the competitor. Thompson and Harper (1988) carried out an experiment in which *T. repens* clones were again grown either without shade (control treatment), under black polythene "leaf" canopies, which cast a neutral shade, or under shade from leaves of one of the three

grass species *Lolium perenne*, *Agrostis tenuis* or *Holcus lanatus*. Canopy densities were manipulated so that all shaded clones of *T. repens* received PAR levels that were significantly lower than those reaching the control clones, but not significantly different from each other. Once again, the frequency of branching along primary stolons was substantially reduced in clones grown under all shade treatments. While the grass canopies cast as much shade as the polythene "leaves," they also substantially reduced the R/FR ratio of light incident on the *T. repens* clones (Table 2); the ratio was almost halved under both *Agrostis* and *Lolium*, compared with the unshaded and neutral shade treatments, and reduced by nearly 80% beneath *Holcus*. The frequency of branching along primary stolon nodes in *T. repens* showed a progressive reduction as R/FR ratio fell ($P<0.02$, $n = 3$), until no branching occurred below the *Holcus* canopy.

Table 1. Mean results from three experiments of Solangaarachchi and Harper (1987) on *Trifolium repens* started on 30 August 1984, 11 October 1984, and 20 June 1985, respectively. Results from each experiment are shown in the three lines under each heading below. Data for total node number were collected from the last experiment only. Within each experiment, those values for the R/FR ratios which are followed by the same letter are not significantly different ($P<0.05$).

	Control	Polythene "Leaves"		Clover Leaves	
PAR Level	100%	50%	30%	50%	30%
R/FR ratio	0.83a	0.84ad	0.85d	0.70b	0.63c
	0.84a	0.87a	0.84a	0.74b	0.64c
	0.87a	0.92a	0.91a	0.66b	0.55c
Number of nodes on primary stolon	10.63	8.75	8.25	8.50	6.50
	13.75	11.33	10.56	11.38	10.25
	13.83	12.44	10.79	11.80	10.11
Number of branched nodes on primary stolon	7.63	3.63	2.38	2.25	0.38
	10.66	5.41	2.94	4.19	2.00
	10.90	8.42	6.64	8.02	4.22
Ratio: branched nodes/ nodes on primary stolon	0.72	0.41	0.29	0.26	0.06
	0.78	0.48	0.28	0.37	0.20
	0.79	0.68	0.62	0.68	0.42
Total number of nodes on entire clone	75.28	52.71	28.06	33.86	18.90
Internode lengths (cm)	2.12	1.76	1.61	1.65	0.91
	2.20	1.69	1.26	1.74	1.49
	2.15	2.39	2.43	2.46	2.41
Petiole lengths (cm)	9.94	9.93	10.27	11.04	10.30
	10.81	10.77	10.92	12.35	13.16
	10.57	13.73	14.22	15.20	17.03

While branching frequency was seen to decline under low PAR and reduced R/FR ratios in both of these experiments, a tendency towards longer internodes, which is a common reaction to such treatments in species with orthotropic shoots (Smith, 1982), was not seen (e.g., Table 1). Internodes were in fact frequently shorter when PAR was reduced, and either showed no consistent change or declined in length in response to lower R/FR ratio. It appears that *T. repens* has a very limited capacity to escape areas of low light availability through

plastic changes that promote the lateral movement of growing apices. However, the lengths of the vertical petioles increased significantly under low PAR, and this effect was more pronounced when low PAR was combined with a low R/FR ratio (Table 1). Thompson and Harper (1988) propose that such plasticity in petiole length may increase the probability that leaves of *T. repens* will be placed above the leaves of competitors in a sward, and suggest that the petiole elongation may be achieved at the expense of resource allocation to stolon branching and internode elongation.

The phytochrome photoequilibrium is known to be extremely sensitive over the range of values for the R/FR ratio found under leaf canopies (Smith, 1982). It therefore has great potential as a cue for shade quality assessment by the plant, enabling the appropriate plastic response to be elicited. In the experiment just described the lower the R/FR ratio, the less stolon branching was displayed in *T. repens*, but clone biomass also declined as the R/FR ratio fell, demonstrating that *T. repens* suffers more from competitors with leaves which transmit a low R/FR ratio. Turkington and Harper (1979) and Solangaarachchi (1985) had previously reported that the growth of *T. repens* is suppressed more by *Holcus* than by other grasses that have been studied. It seems probable that the cause is the low R/FR ratio that *Holcus* transmits, as suggested by Thompson and Harper (1988). It is important to note that the impact of shading by *Holcus* upon node production by *T. repens* is far less than its effect upon node branching; compared with control clones, those under *Holcus* reduced node production on primary stolons by approximately 60%, whereas node branching was completely eliminated (Table 2).

Table 2. Mean results from Thompson and Harper (1988) on the effects of neutral shading by black polythene "leaves," and shade with an altered R/FR ratio, on growth and form in *Trifolium repens*. Shade with an altered R/FR ratio was produced by canopies of *Agrostis tenuis*, *Lolium perenne*, and *Holcus lanatus*. Results tabulated are for primary stolons only.

	Control	Polythene	*Agrostis*	*Lolium*	*Holcus*
R/FR ratio	1.00	1.02	0.56	0.55	0.22
Mean number of nodes	17.30	14.20	11.50	11.20	6.80
Mean number of branched nodes	15.00	9.50	1.60	0.90	0.00
Ratio of branches/nodes	0.87	0.67	0.14	0.08	0.00

Several recent publications have reported the capacity for light reflected from adjacent plants, and even from plants at some distance, to cause alterations in plant morphology (Ballaré et al., 1987; Ballaré, Scopel, and Sánchez, 1990; Casal, Sánchez, and Gibson, 1990). It has been proposed that this reflected light can be used as a cue signalling impending competition for light well before the neighboring plants actually intercept light. Again, the morphological response appears to be induced by a reduction in the R/FR ratio in the reflected light compared with direct sunlight. Previous illustrations of this phenomenon have all come from work on grasses, or species with prostrate forms with a single rooting point. Marcuvitz and Turkington (unpublished) are conducting a series of experiments in which *T. repens* is growing in the vicinity of *Dactylis glomerata* in such a way that the grass does not impede the passage of light to the *T. repens*, but its light-reflection characteristics can act as a warning signal of impending competition for light. Preliminary results indicate that in comparison

with control clones there is no response in branching and no change in either the proportion of stolon nodes producing branches, or the age (measured in plastochrons) at which branch production commences. However, other variables such as petiole length, petiole orientation, and lamina area appear to be responding to the proximity of *Dactylis*. Thus, neighbors that have as yet little direct competitive effect may provoke morphological preparations in plants that could limit the impact of competition when it becomes direct.

Experiments by Kemball, Palmer, and Marshall (1992) on the effects of neutral shade and imposition of darkness upon the morphology of *T. repens* reveal strong integration in this species, with adverse environmental conditions experienced by one part of the clone provoking changes in the pattern of assimilate distribution that promote survival of the disadvantaged part. All the experiments were conducted on clones of a standard size and structural complexity. At the beginning of the experiment they consisted of a primary stolon having eight nodes, and a number of secondary, and smaller tertiary level stolon branches (Figure 1a). The most proximal stolon branch (B1, Figure 1a) was prevented from rooting at its nodes. This stolon was either unshaded (control clones), subjected to 80% neutral shade or to complete darkness. After 35 days of treatment, measurements were made of stolon length, number of nodes, and number of nodes from which lateral buds grew, on the primary stolon and stolons B1 and B2 (Figure 1a).

Neither shading the B1 stolon by 80% nor placing it in darkness significantly affected elongation or node production by the primary, B1 or B2 stolons. Branching of nodes on the primary stolon was not affected by either shading treatment, but branching of the B1 stolon was reduced by shading, and almost eliminated in the dark treatment. However, these stolons survived both treatments for 35 days, although in the dark treatment their biomass was 79% less than that of the equivalent stolons on control clones. Neither shade nor imposition of total darkness upon the B1 stolon affected the growth of other parts of the clone. The development of the clones was therefore very resilient during the long experimental period for which strongly unfavorable conditions were imposed.

Kemball, Palmer, and Marshall (1992) explained this resilience by analyzing the patterns of distribution of assimilates within the clone, using ^{14}C. The B1 stolon imported ^{14}C from elsewhere on the clone in all treatments including the control, but import was unaffected by treatment. However, shaded and darkened B1 stolons exported less assimilate to other parts of the clone than B1 stolons of control clones (92% vs. 78% export in the control and shaded treatments respectively, $P<0.05$). Instead, more assimilate moved acropetally from the oldest ramets on these stolons towards the stolon apex. Thus, while the whole clone is extensively integrated, with assimilate moving between stolons, shading of one stolon results in enhanced retention of assimilate by that stolon, which sustains its own survival and development.

MORPHOLOGICAL RESPONSES OF *TRIFOLIUM REPENS* TO THE PRESENCE OF *RHIZOBIUM TRIFOLII*

Whereas competitors, by reducing light availability and changing light quality, cause a reduction in growth and branching frequency in *Trifolium repens*, the presence of another biotic factor, namely the nitrogen-fixing bacterium *Rhizobium trifolii*, has a positive effect on both of these attributes. Shivji and Turkington (1989) analyzed the morphological consequences of inoculation with this bacterium in an experiment designed to expose half of individual *T. repens* clones to soil containing *R. trifolii* while keeping the other connected half in sterilized soil, from which the bacterium was absent. Clones were arranged so that two stolon branches from a single main stolon grew into two separate flats of soil, one of which contained the bacterium.

A similar treatment was established in which the halves of the clones were severed from each other. Comparison of the results from the two treatments allowed assessment of whether a connection between parts of clones growing in the presence and absence of *R. trifolii* caused a change in growth form, due to physiological integration. It has been suggested (Harvey, 1970; Hartnett and Bazzaz, 1983, 1985; Schmid et al., 1988) that physiological connections between parts of clones growing in different conditions may allow the genet to integrate local variation in resource availability, and that this may be expressed throughout the clone as a compromise between the different morphologies that would be displayed by isolated parts of the clones in each growing condition.

Comparisons between the growth of parts of clones in the presence of *R. trifolii* and parts growing in its absence, and whether or not they were connected, showed that several characters, including the percentage of the main stolon nodes that branched, reached significantly greater values in the presence of *R. trifolii* (Table 3). Benefits accruing from growing with *R. trifolii* included production of nearly 50% more ramets, occupation of nearly 60% more area, and accumulation of 60% more above ground biomass. At the same time, physical connection between clone halves caused no significant difference in any measured characters. Thus, at the structural scale at which the results of this experiment were analyzed, there was no evidence of physiological integration altering growth or morphology when the two halves of the clone were connected; each half developed a morphology that was purely a response to its local growing conditions.

Table 3. Mean results from an experiment of Shivji and Turkington (1989) on clones of *Trifolium repens*. Half of each clone was inoculated with *Rhizobium trifolii*, while *Rhizobium* was absent from the other, connected half of the same clone. Level of significance (or n.s.—not significant) indicated in parentheses.

	Rhizobium present	*Rhizobium* absent	
Number of ramets	92.1	62.4	($P<0.014$)
Length of main stolon (cm)	37.7	32.0	(n.s.)
Total length of all stolons (cm)	124.6	74.5	($P<0.008$)
Area occupied by clone (cm^2)	526.8	334.5	($P<0.035$)
Shoot dry weight (g)	2.02	1.26	($P<0.006$)
Internode length (cm)	1.97	1.97	(n.s.)
Percentage of nodes that branched (main stolon only)	71.8	56.2	($P<0.010$)

MORPHOLOGICAL RESPONSES OF *GLECHOMA HEDERACEA* TO HETEROGENEOUS RESOURCE SUPPLY

The studies of Kemball, Palmer, and Marshall (1992) imply that the carbon economy of different stolons in *T. repens* clones is capable of substantial integration, whereas the results of Shivji and Turkington (1989) show that analysis of form and biomass production provide no evidence for integration between connected halves of clones. The latter conclusion is more similar to the situation in *Glechoma hederacea*. Detailed study of vascular anatomy in *G. hederacea* and its relation to the insertion of leaves in its four orthostichies (Figure 1c; leaves on a common longitudinal line are said to lie in the same orthostichy) has enabled the patterns of integration and independence between leaves and ramets to be explained by a simple set of rules that depend upon vascular continuities and discontinuities (Price, Marshall, and Hutchings, 1992; Price and Hutchings, 1992): (i) leaves in opposite orthostichies and their axillary stolons are largely physiologically isolated; (ii) leaves in adjacent orthostichies

and their axillary stolons are potentially partially integrated; (iii) leaves in the same orthostichy and their axillary stolons are potentially fully integrated; (iv) as an axillary bud develops into a stolon it captures most of the resources translocated acropetally from the subtending leaf, diverting them from leaves in the same orthostichy on the original stolon; and (v) when the new stolon can support its own development, it retains most of the resources it acquires, hardly exporting any to other stolons, thus achieving the status of a new IPU. The first three rules only operate when ramets are on the same structural branch of the clone, because of the predominance of acropetal translocation in the stele (Price, Marshall, and Hutchings, 1992).

An understanding of the physiological organization of *G. hederacea* enables experimental observations to be clearly interpreted. Slade and Hutchings (1987c) conducted an experiment that simulated the growth of clones of *G. hederacea* from a habitat patch with one set of growing conditions into a second patch. In some treatments the second patch was of the same quality as the first, whereas in others the quality differed. The treatments involved availability of light (L) and nutrients (N) as two habitat variables, each of which could be supplied in either high (+) or low (-) amounts (Table 4). The combinations of patch types used in the experiment were limited by the rule that the supply of at most one variable was to change as the clone moved between patches. It had previously been established that, at these levels of supply, patch quality for growth of *G. hederacea* declined in the order +L+N, +L-N, -L+N. Clones were grown from uniform-sized single ramets excised from monoclonal material. One primary stolon grew from the axil of each of the two leaves of this parent ramet, and as each daughter ramet developed from these stolons it was rooted individually in a pot of soil with the appropriate nutrient level (either +N or -N) placed in the appropriate light environment (either +L or -L).

Table 4. Mean results from an experiment in which *Glechoma hederacea* clones commenced growing in either high light and high nutrient conditions (+L+N), high light and low nutrient conditions (+L-N) or low light and high nutrient (-L+N) conditions. After each clone had produced six ramets on each of its primary stolons, it was treated as if it had grown across a habitat patch boundary, and entered conditions of either +L+N, +L-N or -L+N. Growth was continued in the new patch until each primary stolon had produced another six ramets. The following results show, for the first and second parts of the clones, the ratio [stolon branches produced (secondary and tertiary branches summed)/ number of primary ramets produced]. Data from Slade and Hutchings (1987c).

First Part of Clones			Second Part of Clones		
+L+N	+L-N	-L+N	+L+N	+L-N	-L+N
2.33			4.00		
2.33				3.50	
2.17					1.17
	2.17		3.83		
	1.83			1.67	
		0.50	2.33		
		0.67			0.00

It is important to note that the rate of production of ramets by the two primary stolons of the clones was identical in all treatments throughout the experiment. As a result it was possible to grow the clones in the first patch type for a standard length of time, at the end of which they had all produced six ramets on all of their primary stolons. All subsequently produced primary ramets were subjected to the conditions appropriate for growth in the second patch, and growth was continued until each of the primary stolons of all clones had produced six more ramets. The experiment was then stopped simultaneously for all clones. The results

presented in Table 4 show the ratio of the number of stolon branches produced per primary ramet, for the parts of the clones growing in the first and second patches (Table 4). As the number and age distribution of the primary ramets was uniform across the experiment, variation in this ratio is principally a consequence of growing conditions rather than of the ramet age at which stolon branches are initiated.

Two aspects of the results are relevant to this discussion. Firstly, branching, both in the first and second parts of the clones, was most abundant in clones grown in +L+N. Progressively poorer branching occurred in +L-N and -L+N. This can be seen by comparing the branching ratios for the first part of clones which began growth in +L+N, +L-N, and -L+N (Table 4), and comparing the ratio for clones starting growth in the same conditions, for example +L+N, and moving into +L+N, +L-N or -L+N patches; as quality of the second patch declines, so does branching ratio in the second half of the clones (from 4.00 to 3.50 to 1.17).

The second important aspect, also illustrated by the last values quoted, is that branching in poor growing conditions in the second patch cannot be fully sustained by the clone having started growth under good (e.g., +L+N) conditions. In other words, acropetal translocation of resources from the part of the stolon in good conditions to the part in poor conditions cannot prevent an alteration in morphology, including a substantial change in the profusion of clone branching. The part of a stolon growing under resource-poor conditions shows more unidirectional growth. Adoption of such a growth form has benefits that include faster passage across the poor quality patch and less investment in ramet establishment within it. However, there is clearly some modification of branching in the second part of the clone as a result of the conditions under which the first part of the clone is growing. For example, when the second part of the clone is growing in a high quality (+L+N) patch, its branching ratio is 4.00 if its first part is growing in a +L+N patch, 3.83 when the first part is in +L-N and only 2.33 when the first part is in -L+N. This is strong evidence that acropetal movement of resources within the stolons has a carryover effect, preventing local expression of a morphology that is a pure response to the specific conditions of the second patch.

These results show the importance, at a within-clone scale, of local environmental conditions shaping morphology. They also provide morphological evidence of within-stolon integration, which affects the morphology in the youngest parts of the stolon. While this may have the disadvantage of preventing young parts of the clone from developing the ideal morphology for their local growing conditions, this disadvantage is presumably outweighed by the advantage of provision of support to stolon apical regions from established parts of the clone in favorable habitat patches. If this were not the case it is likely that stolon fragmentation would be the evolved response, leading to probable death of parts in poor habitat patches, and release from apical dominance of the axillary buds on the surviving, basipetal part of the stolon. In most species the outgrowth of such axillary buds follows a different direction from that of the apex, raising the likelihood that the same poor quality habitat patch would not be entered again (Headley, Callaghan, and Lee, 1988).

While acropetal translocation of resources along individual stolons (essentially a manifestation of parental care that aids the establishment of young ramets) is commonly observed in stoloniferous plants, information about virtual independence of stolons, both in resource economy and morphological development, is more unusual. One vivid illustration of this phenomenon, which allows some comparisons to be drawn with results already presented for *Trifolium repens*, comes from an experiment on the effects of competition upon *G. hederacea* morphology (Price and Hutchings, unpublished). Clones bearing two primary stolons were grown from single, uniform-sized ramets excised from a monoclonal source. Ramets were established individually in pots, either without competition, or in competition

with either short (1cm) or tall (8–13cm) grass *(Lolium perenne)*, and grown for twelve weeks. These treatments are referred to below as pure treatments. Two additional treatments (split treatments) were established. In these, all ramets on one stolon of a clone were subjected to competition with either short or long grass while those on the other stolon were grown without competition. Both PAR and the R/FR ratio of light reaching the stolon nodes were significantly reduced by the short grass treatment, and reduced further in the long grass treatments. Thus both of these treatments involved competition for soil-based resources and light, but competition for light was significantly greater in the tall grass treatment.

The rate of primary ramet production was once again constant across all experimental treatments, enabling the extent to which clones branched to be interpreted as simple responses to growing conditions, rather than being complicated by variable ramet age structures. No tertiary stolons were produced in this experiment, so that the branching ratios calculated for each stolon (Table 5) would have a maximum value of 2.0 if all primary ramets produced a secondary stolon from the axil of each of their two leaves.

As in *T. repens*, branching declined when clones of *G. hederacea* were subjected to low PAR and low R/FR ratios; the effect was greater when both PAR and R/FR were lower (Table 5). The results also show that, at the structural level used in these experiments, the branching ratios for each individual stolon in the split treatments almost exactly match those found in the corresponding pure treatment clones. Under the homogeneous conditions that it experiences in this experimental design, each stolon of the split treatment clones produces a pattern of branch development that is a direct and pure response to local growing conditions—the most advantageous morphology (Turkington, Sackville Hamilton, and Gliddon, 1991) for its local growing conditions. It is not affected by supply of, or demand for, resources from elsewhere on the connected clone structure. Such an organization allows stolon branches in high-quality habitat to maximize their rate of growth without the reduction involved when support is provided to other parts in poor-quality habitat. At the same time, stolons in poor-quality habitat develop the most advantageous morphology to maximize their probability of escape from this habitat, by linear growth (Hutchings and Mogie, 1990).

Table 5. Mean results from an experiment of Price and Hutchings (unpublished) in which each of the two stolons on *Glechoma hederacea* clones were grown either without competition or with competition from either long or short grass. The experimental design allowed each of the two stolons on a given clone to be subjected to different levels of competition. The table shows for each stolon the ratio of number of secondary stolons to number of primary ramets.

No Competition	No Competition	Short Grass	Short Grass	Long Grass	Long Grass
1.75	1.63				
1.76					
1.72		1.06			
				0.28	
		1.06	1.14		
				0.29	0.26

CONCLUSIONS

Trifolium repens and *Glechoma hederacea* differ both in their physiological organization and ecological behavior. *Trifolium repens* establishes ramets that persist, at least as structures with functional roots, for a considerable time. The ramets remain valuable in resource acquisition, and an integrated physiological organization is employed to support their survival and maintenance of site occupation within the sward even under low light cond-

itions (Kemball, Palmer, and Marshall, 1992). In contrast, *G. hederacea* moves through its habitat at high speed, establishing ramets in preemptive strikes on microhabitats with abundant resources or low competition, but vacating these sites as resource abundance declines or competition intensifies. This fast exploration or exploitation of habitat can be achieved most effectively by rapid deployment of structural branches that are virtually independent in physiological terms, bearing integrated strings of short-lived ramets (Slade and Hutchings, 1989).

The studies described in this paper reveal dramatic plasticity in branching probability, both at the level of complete clones and clone parts, in response to the type of variability in growing conditions that clonal species will usually encounter as they spread laterally throughout their habitats. Undoubtedly the examples presented here are echoed in many other species, including non-clonal ones. They serve as a reminder that this easily recorded morphological parameter has little taxonomic significance.

LITERATURE CITED

Ballaré, C. L., R. A. Sánchez, A. L. Scopel, J. J. Casal, and C. M. Ghersa. 1987. Early detection of neighbor plants by phytochrome perception of spectral changes in reflected sunlight. Pl. Cell Environm. 10: 551–557.

_____, A. L. Scopel, and R. A. Sánchez. 1990. Far-red radiation reflected from adjacent leaves: An early signal of competition in plant canopies. Science 247: 329–332.

Bell, A. D. 1984. Dynamic morphology: A contribution to plant population ecology. Pp. 48–65 *in* R. Dirzo and J. Sarukhán (eds.), Perspectives on Plant Population Ecology. Sinauer, Sunderland, MA.

Casal, J. J., R. A. Sánchez, and D. Gibson. 1990. The significance of changes in the red/far-red ratio, associated with either neighbor plants or twilight, for tillering in *Lolium multiflorum* Lam. New Phytol. 116: 565–572.

Chapman, D. F., M. J. Robson, and R. W. Snaydon. 1991. Quantitative carbon distribution in clonal plants of white clover (*Trifolium repens*): Source-sink relationships during undisturbed growth. J. Agric. Sci. (Cambridge) 116: 229–238.

_____, M. J. Robson, and R. W. Snaydon. 1992. Physiological integration in the clonal perennial herb *Trifolium repens* L. Oecologia 89: 338–347.

Franco, M. 1986. The influence of neighbors on the growth of modular organisms with an example from trees. Philos. Trans., Ser. B 313: 209–225.

Hardwick, R. C. 1986. Physiological consequences of modular growth in plants. Philos. Trans., Ser. B 313: 161–173.

Hartnett, D. C. and F. A. Bazzaz. 1983. Physiological integration among intraclonal ramets in *Solidago canadensis* L. Ecology 64: 779–788.

_____ and _____ . 1985. The integration of neighborhood effects by clonal genets in *Solidago canadensis* L. J. Ecol. 73: 415–427.

Harvey, H. J. 1970. Patterns of assimilate translocation in *Trifolium repens*. Brit. Grassland Soc. Occas. Symp. 6: 181–186.

_____. 1979. The regulation of vegetative reproduction. Ph.D. Thesis, University of Wales.

Headley, A. D., T. V. Callaghan, and J. A. Lee. 1988. Water uptake and movement in the clonal plants, *Lycopodium annotinum* L. and *Diphasiastrum complanatum* (L.) Holub. New Phytol. 110: 497–502.

Hoshino, M. 1974. Translocation and accumulation of assimilates and phosphorus in Ladino clover. Bull. Natl. Grassland Res. Inst. 5: 35–84.

Hutchings, M. J. and M. Mogie. 1990. Phylogeny, ontogeny, and clonal growth in vascular plants. Pp. 3–22 *in* J. van Groenendael and H. de Kroon (eds.), Clonal Growth in Plants: Regulation and Function. SPB Academic Publishing, The Hague.

Jones, M. and J. L. Harper. 1987a. The influence of neighbors on the growth of trees. I. The demography of buds in *Betula pendula*. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 232: 1–18.

_____ and _____. 1987b. The influence of neighbors on the growth of trees. II. The fate of buds on long and short shoots in *Betula pendula*. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 232: 19–33.

Kemball, W. D., M. J. Palmer, and C. Marshall. 1992. The effect of local shading and darkening on branch growth, development, and survival in *Trifolium repens* and *Galium aparine*. Oikos 63: 366–375.

Meicenheimer, R. D. 1981. Changes in *Epilobium* phyllotaxy induced by N-1-naphthylphthalamic acid and α-4-chlorophenoxyisobutyric acid. Amer. J. Bot. 68: 1139–1154.

Newton, P. C. D. 1986. The establishment, growth, and fate of white clover plants with special reference to the physiology of stolon growth. Ph.D. Thesis, University College of North Wales, Bangor.

Price, E. A. C. and M. J. Hutchings. 1992. The causes and developmental effects of integration and independence between different parts of *Glechoma hederacea* clones. Oikos 63: 376–386.

_____, C. Marshall, and M. J. Hutchings 1992. Studies of growth in the clonal herb *Glechoma hederacea*. I. Patterns of physiological integration. J. Ecol. 80: 25–38.

Schmid, B., G. M. Puttick, K. H. Burgess, and F. A. Bazzaz. 1988. Clonal integration and effects of simulated herbivory in old-field perennials. Oecologia 75: 465–471.

Shivji, A. and R. Turkington. 1989. The influence of *Rhizobium trifolii* on growth characteristics of *Trifolium repens*: Integration of local environments by clonal genets of *T. repens*. Can. J. Bot. 67: 1080–1084.

Slade, A. J. and M. J. Hutchings. 1987a. The effects of nutrient availability on foraging in the clonal herb *Glechoma hederacea*. J. Ecol. 75: 95–112.

_____ and _____. 1987b. The effects of light intensity on foraging in the clonal herb *Glechoma hederacea*. J. Ecol. 75: 639–650.

_____ and _____. 1987c. Clonal integration and plasticity in foraging behaviour in *Glechoma hederacea*. J. Ecol. 75: 1023–1036.

_____ and _____. 1989. Within- and between-population variation in ramet performance in the gynodioecious clonal perennial herb *Glechoma hederacea*. Can. J. Bot. 67: 633–639.

Smith, H. 1982. Light quality, photoperception, and plant strategy. Ann. Rev. Plant Physiol. 33: 481–518.

Solangaarachchi, S. M. 1985. The nature and control of branching pattern in *Trifolium repens*. Ph.D. Thesis, University of Wales.

_____ and J. L. Harper. 1987. The effect of canopy filtered light on the growth of white clover *Trifolium repens*. Oecologia 72: 372–376.

Thompson, L. and J. L. Harper. 1988. The effect of grasses on the quality of transmitted radiation and its influence on the growth of white clover *Trifolium repens*. Oecologia 75: 343–347.

Turkington, R. and J. L. Harper. 1979. The growth, distribution, and neighbor relationships of *Trifolium repens* in a permanent pasture. IV. Fine-scale biotic differentiation. J. Ecol. 67: 245–254.

_____ R. Sackville Hamilton, and C. Gliddon 1991. Within-population variation in localized and integrated responses of *Trifolium repens* to biotically patchy environments. Oecologia 86: 183–192.

Watson, M. A. and B. B. Casper 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Ann. Rev. Ecol. Syst. 15: 233–258.

PLANT REPRODUCTIVE STRATEGIES: USING THE FOSSIL RECORD TO UNRAVEL CURRENT ISSUES IN PLANT REPRODUCTION[1]

David L. Dilcher[2]

ABSTRACT. The evolution of angiosperm flowers and fruits including modifications of individual organs of flowers is directly related to the potential of these organs to influence the genetic composition of early angiosperm populations. The closure of the carpel, the reduction and elevation of the stigma, and changes in the size and positioning of flowers are all features that developed very early in floral evolution. For the most part, these were modifications related to the early development of insect pollination in the angiosperms, probably during the Lower Cretaceous, approximately 120 million years ago. By the Eocene, about 50 million years ago, angiosperms had developed floral bilateral symmetry and edible fruits and nuts. These two developments are evidence for the coevolution of angiosperms with social insects, birds, and rodents. Also in the Eocene, wind dispersal of angiosperm fruits became common, demonstrating that abiotic factors were also an influence in the evolution of angiosperms. The evolution of closed carpels, reduced and elevated stigmatic areas, flower position and size, followed later by the evolution of bilateral symmetry, biotic and abiotic dispersal of fruits and seeds, are all factors that increased the potential for outcrossing. These characters strongly suggest that the tendency towards outcrossing has been a driving force in angiosperm evolution.

Investigations into the phylogeny of angiosperms should be grounded on the fossil record. When this is not possible, inferences about angiosperm phylogeny based on a variety of molecular and morphological tools of investigation can only be viewed as indirect evidence awaiting further confirmation from the fossil record. These tools of investigation include analysis of the plastid *rbc*L gene, cladistics, phytogeography, and comparisons of the anatomy and morphology of particular organs of extant angiosperms. The fossil record of angiosperm reproduction and its changes through time is full of gaps and incomplete data. However, much better information is available now than was available just a few years ago, and this paper presents some of this information as it bears upon the evolution of angiosperm reproduction.

For the most part, the systematics of extant flowering plants has been based on differences in the gross features of their reproductive organs. These organs occur during a critical stage in the life cycle of angiosperms when the possibilities for limiting the continuation of particular genetic lines are extremely high. The success of pollination, fertilization, fruit set, seed dispersal, seed germination, and seedling survival are essential for the genetic continuity of a taxon. This reproductive time is the major phase in the life cycle of

[1]The ideas raised in this paper are the products of and influenced by, various contacts that I have had with colleagues who work on fossil plants and those who work with living plants. I thank Andrew Stephenson for his invitation and encouragement to contribute this paper. The comments of Victor Call, Michael Muller, Steven Manchester, Conrad Labandeira, and Katherine Dilcher in reviewing this manuscript are appreciated. The help of Terry Lott in the preparation of this paper is appreciated. The help of several artists with the preparation of the reconstructions illustrated is acknowledged: Figures 1 and 2 were drawn by Megan Rohn, Figures 4 and 5 by Bronwyn Elkuss, Figure 3 by Wendy Zomlefer, and Figure 6 by Sally Wolfe. Paper number 425 of the Series, University of Florida Contributions to Paleobiology.

[2]Department of Natural Science, Florida Museum of Natural History, Gainesville, FL 32611, U.S.A.

a plant when new genetic combinations are made. Fruits and seeds are dispersed, which tends to promote future genetic mixing. The evolution of mechanisms that facilitate genetic mixing, particularly outcrossing, has been one of the major driving forces in the reproductive biology of land plants throughout their history. The importance of outcrossing is central to the early evolution of both floral and fruit structures observed in the fossil record. There are two basic nodes of evolution in the angiosperms that are tied directly to plant/animal coevolution. The first of these is the evolution of the flower and associated floral organs. The second node of evolution is modification of fruits and seeds. Both of these nodes of evolution are centered around the importance of outcrossing in the success and continued radiations of the flowering plants.

The earliest generally accepted reproductive remains of flowering plants are found in the Lower Cretaceous, although numerous reports of earlier angiosperms and angiosperm-like reproductive organs have been made (Krassilov, 1989; Cornet, 1989). These need to be investigated further before they can be confirmed. While the evolution of the angiosperms probably began before the Lower Cretaceous, the fossil record discussed in this report will be limited primarily to some Lower and mid-Cretaceous and some Eocene fossils selected to illustrate the points raised. Many early flowering plant reproductive remains have been reported to be fossil flowers when in fact they are fruiting remains. This is the case with many, if not most, so-called fossil flowers of early angiosperms; few direct observations have been made of the often fragile and deciduous organs of early flowers. However, some true flowers with petals and stamens have been reported (Basinger and Dilcher, 1984; Muller and Dilcher, 1991; Drinnen et al., 1990). Fruiting material is also important in phylogenetic considerations and can provide insights about the reproductive biology of early flowering plants.

Coevolution has been a major aspect of plant evolution. Coevolutionary history goes back at least to the incorporation of the chloroplast and the mitochondria as organelles in the eukaryotic cell over 1,500 million years ago. Another major coevolutionary step was the evolution of mycorrhizal fungi, probably associated with the early evolution of vascular land plants during the Silurian and early Devonian (Pirozynski and Malloch, 1975). Certainly by the Coal Ages, about 350 million years ago, some seed plants had developed a reproductive biology that probably was aided by insects as a vector for pollen transfer. For example, *Medullosa*, a seed fern, produced pollen so large that it is unlikely that it could have been effectively carried by wind; more likely, it was transported by insects (Dilcher, 1979; Taylor and Taylor, 1992).

Animal pollination of seed plants was independently acquired by other extinct groups of plants during the Mesozoic. One example is found in the cycadeoid *Williamsonia* from Jurassic deposits of Mexico (Delevoryas and Gould, 1973). These plants had large, well-developed, and symmetrically arranged bracts surrounding an ovule-bearing receptacle. These conspicuous bracts probably served as showy petal-like structures for attracting insects. Delevoryas (1968) suggested that insects may have played a role in the pollination biology of Upper Cretaceous *Cycadeoidea* "flowers." Another group, cycads, also evolved during the Mesozoic, and some are known to be insect-pollinated today (Norstog et al., 1986). Therefore, plant-animal pollination syndromes were in place in diverse groups of plants before such syndromes became widespread in various angiospermous groups. Other direct and indirect evidence supporting this thesis includes analyses of family-level diversity and insect mouthpart disparity during the Mesozoic and Cenozoic (Labandeira, 1992; Labandeira and Sepkoski, 1993).

Some aspects of the pollination biology of seed plants are important in determining their evolutionary history. Early seed plants had imperfect, or unisexual, reproductive organs,

often bearing several ovules on leaves or organs modified from leaves and having complex pollen organs also associated with leaves. The transfer of pollen to the micropyle in many (perhaps most) Paleozoic and Mesozoic seed plants was accomplished by wind. Wind was and still is an effective agent of pollen transfer for many seed plants, as evidenced in extant conifers. Wind as an abiotic pollination agent has not changed much since the Paleozoic or Mesozoic. It effectively promotes outcrossing, especially when ovules and pollen of the same plant mature at slightly different times.

The pollen grain is the male gametophyte and the nature of its transfer to the ovule is extremely important to the plant. If the transfer of pollen to the ovules of the same plant occurs regularly, inbreeding results, probably causing extinction over extended periods of time. Heterospory promotes outcrossing. The earliest heterosporous plants from the Upper Devonian used wind or water to effect outcrossing (Dilcher, Kar, and Dettmann, 1992; Niklas, 1981). The genetic exchange permitted by abiotic pollination of early seed plants has fostered outcrossing since the late Devonian. However, there are certain advantages to the transfer of pollen by animals, and this mechanism of pollination apparently developed in the Paleozoic (Scott and Taylor, 1983) and again in the Mesozoic, where it was present in various seed ferns, cycads, and cycadeoids. Insect pollination syndromes evolved independently and repeatedly during the Mesozoic. Insect pollination also probably developed in several different groups of flowering plants independently during the Mesozoic and Cenozoic. Because the method of pollination is so important in flowering plants, and because the form of the flower is so strongly influenced by the mode of pollination, many parallel lines of convergent floral morphologies have evolved during geologic time.

FLORAL EVOLUTION

The floral organs that are typical of angiosperm flowers today occur extremely early in the evolution of angiosperms. However, some aspects of early floral organs differ from any known in extant angiosperms (Retallack and Dilcher, 1981; Dilcher and Kovach, 1986; Crane and Dilcher, 1984; Drinnen et al., 1990). While early fruits, fruiting axes, and flowers of extinct Cretaceous flowering plants demonstrate diagnostic angiosperm features (Crepet et al., 1992), the combinations of most of these characters are unknown in angiosperm flowers today.

Particular changes that have taken place during floral evolution can be observed in the fossil record and have resulted in important features of flowers and fruits today. Some of these features include: (1) the closed carpel, (2) reduction and elevation of the stigma, (3) modifications of flower position and size, (4) change in floral symmetry, and (5) modification of the fruit and/or seed for animal and wind dispersal. The development of each of these evolutionary features has occurred differently in various angiosperm groups, and the combined influence of these evolutionary events has resulted in the diversity of flowers, fruits, and seeds that we know today.

The Closed Carpel. The closed carpel is a unique feature of angiosperms. Not all extant flowering plants have completely or tightly closed carpels. For example, those of *Platanus* are loosely closed, and the same is true of *Degeneria*, *Drimys*, Butomaceae, Hydrocharitaceae, *Sparganium*, *Sassafras*, *Coptis*, and *Tiarella* (Eames, 1961). However, there is no indication that these carpels are open enough for pollen grains to enter; the pollen germinates on the outside of the carpel and only the pollen tube enters the carpel. This is important because the pollen tube (which carries the haploid sperm of the male gametophyte) must either penetrate some of the cells of the diploid tissue producing the ovules (which

contain the haploid female gametophyte) or come into very intimate contact with this diploid tissue. The more complete the carpel closure, the more intimate the contact. When such contact takes place, the two tissues interact biochemically to determine compatibility; any incompatibility reaction will stop or limit pollen tube growth (Stephenson and Bertin, 1983; Schlichting et al., 1987). One of the earliest features of the carpel was its closure, although some early fossil carpels, such as a *Ceratophyllum*-like plant and *Platanus*, appear to have had only incompletely or loosely closed carpels. Zavada (1984) argued that the pollen wall ultrastructure of early angiosperms was typical of the pollen wall of those genera that demonstrate incompatibility today. However, there has been some debate in the literature regarding this conclusion (Gibbs and Ferguson, 1987; Zavada, 1990).

Among the earliest known carpels in the fossil record are those from the early Aptian (115 MYA) in the Koonwarra Formation, Victoria, Australia. One Koonwarra plant, described by Taylor and Hickey (1990), has simple, individual carpels arranged helically along very short axes. Each carpel-bearing shoot is borne in a leaf axis and each carpel is subtended by a bract. The carpels are exceedingly small in the fruiting stage, so it is difficult to determine for certain the nature of closure and the location of the stigmatic area; however, the carpels appear to be closed and the stigmatic area limited.

Another carpel from the Koonwarra Formation is the fruit originally called *Hemitrapa* and renamed "undetermined" by Drinnen and Chambers (1986). This appears to be a fruit of a *Ceratophyllum*-like plant that has many similarities to this extant genus. The carpel of extant *Ceratophyllum* lacks a stigma, and the pollen tube, growing underwater, penetrates the carpel through a small pore in the carpel wall. The fossil carpels examined are in the fruiting stage, and the pollination pore is not visible in these ancient fruits. However, since there is no alternative area evident in the fossil fruits through which the pollen tube might have penetrated, it can be assumed that a similar pollination pore existed in these fossils.

Lower and mid-Cretaceous *Platanus* fossils have incompletely closed carpels with stigmas along the adaxial surface. Often the carpels of these fossils are infilled with sediment. This sediment infilling could reflect post-maturation opening of these fruits, but probably indicates that they were never completely closed. Living species of *Platanus* still have poorly fused carpels; they are and probably always have been wind-pollinated (Dilcher, 1979), which appears to promote effective outcrossing. Careful studies demonstrating pollen compatibility relationships or pollen tube penetration of the carpel bearing the young ovule have not been done for living *Platanus*. The results of such studies would be very interesting since Platanaceae may be one of the oldest extant families of angiosperms; if it has a pollen compatibility system similar to that of conifers, it might indicate that it is derived from a unisexual ancestry.

The closure of the carpel was one of the earliest features found in angiosperm bisexual flowers. It has been suggested that it was closed to provide mechanical protection for the young developing ovules. This is especially relevant if we consider that the early pollinators may have been beetles indiscriminately eating any flower parts exposed (Proctor and Yeo, 1972; Crowson, 1981; Gottsberger, 1987). Others have considered that the closure of the carpel enabled the establishment of biochemical control over the entry of the pollen tubes (Taylor and Zavada, 1986). I favor this second hypothesis and suggest that the closure of the carpel was not complete in the earliest unisexual pre-angiospermous ancestral stock. Subsequently, it never closed tightly in at least some of those taxa that remained unisexual and are pollinated underwater or that continued to use wind as a pollination agent.

However, carpel closure was extremely important in those early flowers that became bisexual because of the frequent chance for self pollination. The pressure to develop bisexual

flowers was a direct consequence of the evolution of insect pollination. Each visit of an insect to bisexual flowers could potentially result in successful pollination, fruit set, and seed production, whereas in unisexual flowers, insect pollination would have more uncertain success. The closure of the carpel is a direct consequence of the importance of developing mechanical and biochemical barriers to self pollination in these early bisexual and insect-pollinated angiosperm flowers.

Reduction and Elevation of the Stigma. The primitive form of the carpel in angiosperms is generally considered to have been elongate and conduplicate with elongate stigmas (Eames, 1961; Cronquist, 1988; Weberling, 1989). Several early angiosperms have elongate conduplicate carpels. Some examples from the Lower and mid-Cretaceous are the carpels of *Caloda* (Figure 6; Dilcher and Kovach, 1986), *Archaeanthus* (Figure 1; Dilcher and Crane, 1984), *Lesqueria* (Figure 2; Crane and Dilcher, 1984), "*Platanus*" (Figure 5; Dilcher, 1979), and *Platanocarpus* (Friis et al., 1988). All of these have several free carpels, each of which bears several seeds, and has elongate stigmas present on the adaxial surface. Some are borne in unisexual flowers, while others appear to have been borne in bisexual flowers. Pollen transport may have varied from wind to insect. The stigmatic area in *Archaeanthus* (Figure 1) bears many hairs that may have aided in capturing pollen from the air or from insects. No other special modifications of the stigmatic areas are known.

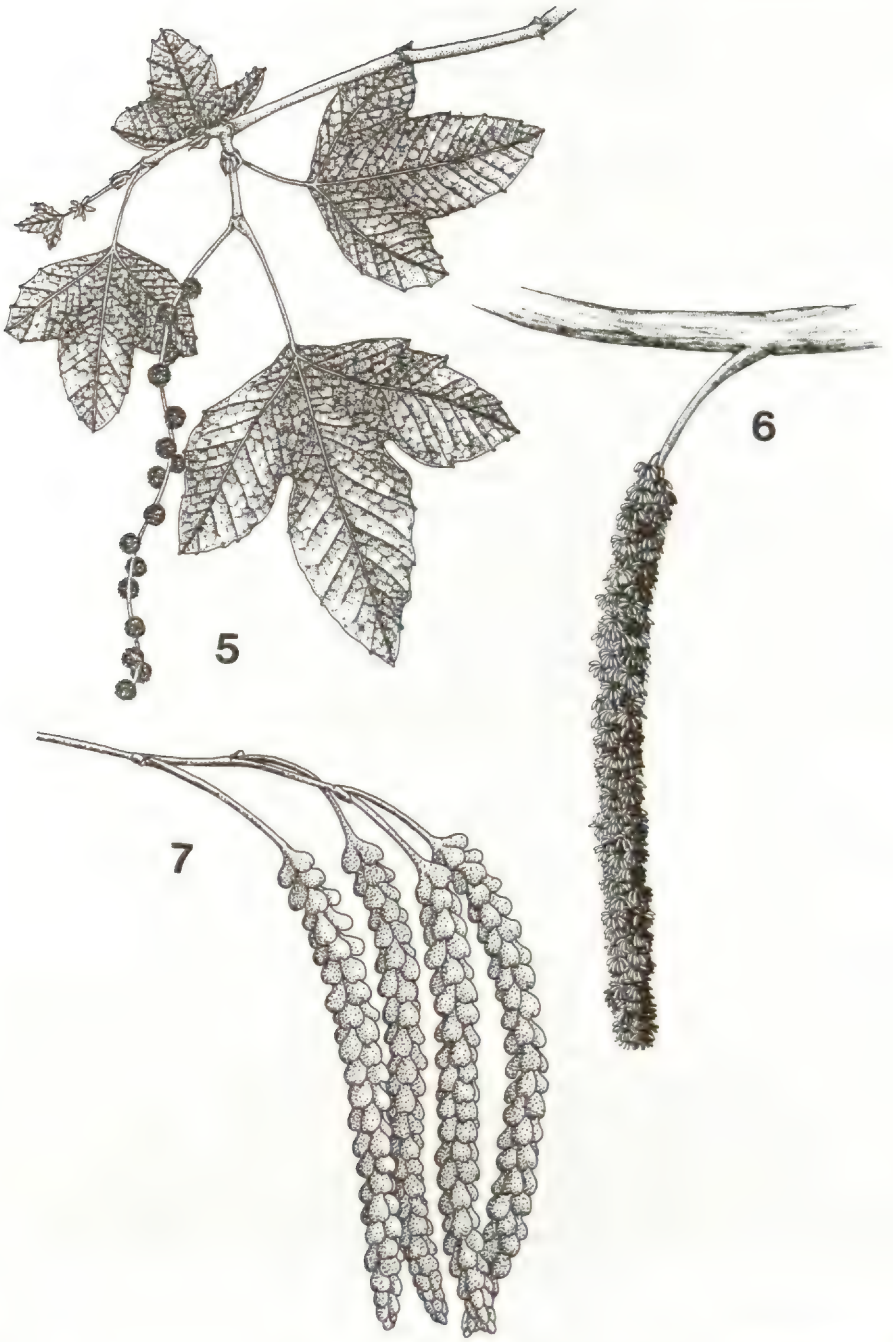
By the mid-Cretaceous some carpels are known to have had restricted stigmatic areas. These are found, among others, in the Rose Creek flower (Figure 4; Basinger and Dilcher, 1984) and a dichasial flower (Figure 3; Muller and Dilcher, 1991). These flowers have carpels that are loosely adnate or fused, containing one or only a few ovules, and have stigmas that were reduced and elevated on a style. These carpels occur in flowers that are pentamerous and apparently have rosid affinities. These flowers are functionally modified for insect pollination, indicating that the pollen load that was left on the reduced and elevated stigmas was probably large and delivered as one event.

These flowers indicate that the potential for pollen-tube competition was already established as early as the mid-Cretaceous, about 100 million years ago. The coevolutionary pollination syndromes that deliver large amounts of pollen to a restricted stigma allow for pollen-tube competition. Such competition would be enhanced if the stigma is elevated (Stephenson and Bertin, 1983; Schlichting et al., 1987). This is exactly what was present in the Rose Creek flower and the dichasial flower. Similar evolution must have been repeated independently in various groups of angiosperms to account for the distribution of this mechanism in extant angiosperm taxa. As floral forms become modified, insects delivered larger pollen loads to restricted pollen receptive areas.

Flower Position. Very early angiosperm flowers from the Koonwarra Formation are small and axillary to leaves. During the mid-Cretaceous, there are large solitary flowers such as *Archaeanthus*, which were probably perfect. These flowers present a large visual impact to potential pollinators by having many parts, including large showy petals, and by being borne terminally on upward-curving branches similar to some magnolias today. This terminal position implies the importance of visual impact for the pollination of these flowers.

Some perfect or probably perfect fossil flowers are much smaller than *Archaeanthus*. The dichasial flower (Muller and Dilcher, 1991) is such an example. The inflorescence consists of numerous flowers arranged in a cyme (Weberling, 1989). The individual flowers are small and clustered in such a way that the petals of each individual flower nearly touches its neighboring flower. This forms a surface over which a pollinator could land, walk, and feed. When several of those small flowers opened at the same time, they would present a

Figures 1–4. Reconstructions of fruiting axes, flowering axes, and a flower that probably all were insect-pollinated. —1. *Archaeanthus*: leafy shoot, bearing numerous follicles that were the product of one large flower. About 1/5 natural size. —2. *Lesqueria*: fruiting axis bearing numerous follicles that were the product of one large flower. About 1/2 natural size. —3. Dichasial cyme, showing several flowers and a few fruits. About 2.1 × natural size. —4. Rose Creek flower, showing a single pentamerous flower. About 1.4 × natural size.



Figures 5–7. Reconstructions of fruiting axes that probably were all wind-pollinated. —5. Platanoid leafy shoot with numerous infructescenses attached to a reproductive axis. About 2/3 × natural size. —6. *Caloda*: fruiting axis bearing hundreds of follicles on small lateral axes. About 3/4 × natural size. —7. *Prisca*: several fruiting axes clustered together. About 1/2 × natural size.

much greater visual impact than was possible by any of the flowers individually. Since not all the flowers in the dichasia flowered at one time, there was a repeated series of flowering by these small simple flowers, coordinated in bursts of blooming that would permit a plant to encourage pollinators to make return visits to a known locality. This will be discussed in more detail in Dilcher and Muller (in progress).

Other flowers in the mid-Cretaceous are clustered together to form circular ball-like (Figure 5) and elongate linear inflorescences/infructescences (Figures 6, 7). Nearly all of these are clusters of unisexual florets lacking elaborated floral organs. These probably were wind pollinated and are represented by such flowers as *Caloda* (Figure 6; Dilcher and Kovach, 1986), *Platanus*, and some pollen-bearing catkins (Dilcher, 1979).

Flower position is directly related to the pollination biology of the plant. This had already affected the diversity of flower types by the mid-Cretaceous. There may have been more diversity in insect-pollinated flowers early in the evolution of flowering plants (Friis and Crepet, 1987) than in wind-pollinated ones. However, more data are needed before this can be determined with certainty.

Flower Symmetry. All of the flowers from the Lower and mid-Cretaceous are radially symmetrical. This is true for those that are small or large and those that are bisexual or unisexual. When the fossil data are taken at face value, this radial symmetry represents a primitive state in ancestral angiosperms. The tendency towards bilateral symmetry can be found during the late Upper Cretaceous (Friis and Crepet, 1987). The earliest clearly bilateral flowers are from the lower Eocene (Crepet and Taylor, 1985; Herendeen, Crepet, and Dilcher, 1992). At that time bilateral symmetry was well developed and was probably related to the availability of bee pollinators. Particular bees act as faithful pollinators (Crepet, 1984), and it is now known that bees were present by the latest Cretaceous (Michener and Grumaldi, 1988). As flowers evolved bilateral symmetry in concert with their faithful pollinators, plants were actually able to direct the behavior of animal pollinators. Bilateral symmetry provides the insect with information about the direction of approach to the flower, where to land, how to enter, and how to proceed to reach the floral reward. Therefore, the flower could exercise greater control through mechanical and biochemical processes for more effective outcrossing.

Bilateral symmetry evolved independently in several lines of flowering plants. Such evolution was not initiated until late in the Cretaceous and no good evidence of well-developed bilateral symmetry is known until the lower Eocene, about 50 million years ago, when it is found associated with some degree of petal fusion. Thus, this cooperation between floral form and pollinator may have developed only in the latter half of the time that angiosperms have been a well-recognized part of global vegetation. This late date when bilateral symmetry evolved is reflected in the variety of groups and different taxonomic levels at which bilateral symmetry can be found in angiosperm flowers today. This is an excellent example of parallel evolution in independent lines of angiosperm taxa. There seems to be no tendency towards the development of bilateral symmetry in wind- or water-pollinated flowers, nor has bilateral symmetry become a major pattern in the floral morphology of any group of land plants other than the angiosperms. Bilateral symmetry is another step towards increasing the potential for outcrossing in the angiosperms.

Fruit Dispersal by Animals and Wind. During the early Tertiary we find evidence for extensive radiation of fruit and angiosperm seed types that appear to have been attractive to animals. Tiffney (1984) pointed out that this diversity coincides with the extensive radiation that took place in birds and mammals (especially rodents) during the late Paleocene and Eocene (Carroll, 1988; Benton, 1990). Here again, the coevolution of animal groups with

plants had a profound and synergistic effect upon both groups of organisms. Whereas pollen transport by animals functioned as an important agent for carrying genes between populations of angiosperms, seed movement has a much more profound effect upon the population structure of angiosperms (Silvertown, 1987). Seed movement further enhanced the potential for outcrossing, and the genetically driven modifications of fruits and seeds became a new arena of change and evolution within angiosperm taxa. The increased potential for outcrossing is important not only for those angiosperms that have animal-dispersed fruits and seeds, but also for the evolution of a variety of wind-dispersal mechanisms (Call and Dilcher, 1992). The Eocene seems to have been a time when new waves of evolutionary innovations for animal and wind dispersal of fruits and seeds overtook the angiosperms, innovations that promoted gene flow via interactions with biotic and abiotic factors that increased fruit and seed dispersal.

One example of such radiation of fruits is the Juglandaceae, in which there are four independent lines of evolution involving animal dispersal (Stone, 1972). There was also a radiation of winged fruits during the Paleocene/Eocene in this family (Dilcher et al., 1976; Manchester, 1987, 1989). The fruits of two genera, *Oreomunnea* (winged) and *Alfaroa* (large-animal dispersed nut), appear to be very different, whereas the wind-pollinated flowers of these two related genera are very similar (Stone, 1972; Manning, 1959). The opposite seems to be true in some genera of the Leguminosae (Dilcher et al., 1992): whereas several genera have similar animal-pollinated flowers, some have evolved wind-dispersed fruits instead of animal-dispersed ones (Gunn, 1984, 1991).

Many aspects of wind pollination and of wind-dispersed fruits and seeds remain relatively constant through time. This is evident in the Leguminosae, *Platanus*, and the Juglandaceae, as well as in many other angiosperm families, primarily because abiotic factors affecting evolution tend to remain constant through time. Biotic factors change much more rapidly through time; therefore, those aspects of the reproductive biology of angiosperms that are dependent upon biotic factors are more subject to change. This is apparent in floral as well as fruit morphology. The underlying force behind all of this change is the tendency for angiosperms to evolve towards systems that promote outcrossing.

CONCLUSIONS

By an examination of the fossil record combined with some understanding of the systematics and the reproductive biology of extant angiosperms, it is possible to interpret some of the features of flowers, fruits, and seeds with an eye towards answering questions about when and why particular reproductive features of the angiosperms might have evolved. Floral features such as the closed carpel, reduced stigma and style, and various changes in floral sizes and positions all are known from the Lower and mid-Cretaceous, early in the evolution of angiosperms. These features appear to be related to interactions with insect pollinators and were important in the reproductive biology of particular angiosperms over 100 million years ago. About 50 million years ago, during the lower Eocene, there is clear evidence for bilaterally symmetrical flowers and a striking increase in animal- and wind-dispersed fruits and seeds. All of these early and subsequent innovations in the reproductive biology of angiosperms represent interactions of angiosperms with biotic and abiotic aspects of their environment. The driving force behind this evolutionary pattern is apparently genetic; most of the features contributing to this pattern are important in promoting outcrossing and gene flow.

LITERATURE CITED

Basinger, J. F. and D. L. Dilcher. 1984. Ancient bisexual flowers. Science 224: 511–513.

Benton, M. J. 1990. Vertebrate Palaeontology. Unwin Hyman, London.

Call, V. B. and D. L. Dilcher. 1992. Early angiosperm reproduction: survey of wind-dispersed disseminules from the Cretaceous-Paleogene of North America. Organisation Internationale de Paléobotanique, IV Conférence, Paris. Abstracts: 36.

Carroll, R. L. 1988. Vertebrate Paleontology and Evolution. W. H. Freeman, New York.

Cornet, B. 1989. The reproductive morphology and biology of *Sanmiguelia lewisii* and its bearing on angiosperm evolution in the late Triassic. Evol. Trends Pl. 3: 25–51.

Crane, P. R. and D. L. Dilcher. 1984. *Lesqueria*: an early angiosperm fruiting axis from the Mid-Cretaceous. Ann. Missouri Bot. Gard. 71: 384–402.

Crepet, W. L. 1984. Advanced (constant) insect pollination mechanisms: pattern of evolution and implications vis-à-vis angiosperm diversity. Ann. Missouri Bot. Gard. 71: 607–630.

_____, K. C. Nixon, E. M. Friis, and J. V. Freudenstein. 1992. Oldest fossil flowers of hamamelidaceous affinity, from the Late Cretaceous of New Jersey. Proc. Natl. Acad. Sci. U.S.A. 89: 8986–8989.

_____ and D. W. Taylor. 1985. The diversification of the Leguminosae: first fossil evidence of the Mimosoideae and Papilionoideae. Science 228: 1087–1089.

Cronquist, A. 1988. The Evolution and Classification of Flowering Plants, 2nd ed. The New York Botanical Garden, New York.

Crowson, R. A. 1981. The Biology of the Coleoptera. Academic Press, London.

Delevoryas, T. 1968. Investigations of North American Cycadeoids: structure, ontogeny, and phylogenetic considerations of cones of *Cycadeoidea*. Palaeontographica, Abt. B, Paläophytol. 121: 122–133.

_____ and R. E. Gould. 1973. Investigations of North American Cycadeoids: Williamsonian cones from the Jurassic of Oaxaca, Mexico. Rev. Palaeobot. Palynol. 15: 27–42.

Dilcher, D. L. 1979. Early angiosperm reproduction: an introductory report. Rev. Palaeobot. Palynol. 27: 291–328.

_____ and P. R. Crane. 1984. *Archaeanthus*: an early angiosperm from the Cenomanian of the western interior of North America. Ann. Missouri Bot. Gard. 71: 351–383.

_____ and W. L. Kovach. 1986. Early angiosperm reproduction: *Caloda delevoryana* gen. et sp. nov., a new fructification from the Dakota Formation (Cenomanian) of Kansas. Amer. J. Bot. 73: 1230–1237.

_____, P. S. Herendeen, and F. Hueber. 1992. Fossil *Acacia* flowers with attached anther glands from Dominican Republic amber. Pp. 33–42 *in* P.S. Herendeen and D.L. Dilcher (eds.), Advances in Legume Systematics: Part 4. The Fossil Record. The Royal Botanic Gardens, Kew.

_____, R. K. Kar, and M. Dettmann. 1992. The functional biology of Devonian spores with bifurcate processes—a hypothesis. Palaeobotanist 41: 67–74.

_____, F. W. Potter, Jr., and W. L. Crepet. 1976. Investigations of angiosperms from the Eocene of North America: juglandaceous winged fruits. Amer. J. Bot. 63: 532–544.

Drinnan, A. N. and T. C. Chambers. 1986. Flora of the Lower Cretaceous Koonwarra fossil bed (Korumburra Group), South Gippsland, Victoria. Pp. 1–77 *in* P. A. Jell and J. Roberts (eds.), Plants and Invertebrates from the Lower Cretaceous Koonwarra Fossil Bed, South Gippsland, Victoria. Association of Australasian Palaeontologists, Sydney.

_____, P. R. Crane, E. M. Friis, and K. R. Pedersen. 1990. Lauraceous flowers from the Potomac Group (Mid-Cretaceous) of Eastern North America. Bot. Gaz. 151: 370–384.

Eames, A. J. 1961. Morphology of the Angiosperms. McGraw-Hill, New York.

Friis, E. M., P. R. Crane, and K. R. Pedersen. 1988. Reproductive structures of Cretaceous Platanaceae. Biol. Skr. 31: 1–55.

_____ and W. L. Crepet. 1987. Time of appearance of floral features. Pp. 145–179 *in* E. M. Friis, W. G. Chaloner, and P. R. Crane (eds.), The Origins of Angiosperms and Their Biological Consequences. Cambridge University Press, Cambridge.

Gibbs, P. E. and I. K. Ferguson. 1987. Correlations between pollen exine sculpturing and angiosperm self-incompatibility systems—a reply. Pl. Syst. Evol. 157: 143–159.

Gottsberger, G. 1987. Some aspects of beetle pollination in the evolution of flowering plants. Pl. Syst. Evol. Suppl. 1: 211–226.

Gunn, C. R. 1984. Fruits and seeds of the genera in the subfamily Mimosoideae (Fabaceae). U.S. Dept. of Agric. Tech. Bull. 1681: 1–194.

_____. 1991. Fruits and seeds of the genera in the subfamily Caesalpinioideae (Fabaceae). U.S. Dept. of Agric. Tech. Bull. 1757: 1–408.

Herendeen, P. S., W. L. Crepet, and D. L. Dilcher. 1992. The fossil history of the Leguminosae: phylogenetic and biogeographic implications. Pp. 303–316 *in* P. S. Herendeen and D. L. Dilcher (eds.), Advances in Legume Systematics: Part 4. The Fossil Record. The Royal Botanic Gardens, Kew.

Krassilov, V. A. 1989. Origin and Early Evolution of Flowering Plants. Nauka, Moscow. [In Russian.]

Labandeira, C. C. 1992. Diversity, diets, and disparity: determining the effect of the terminal Cretaceous extinction on insect evolution. 4th North American Paleontological Convention. Abstracts: 174.

_____ and J. J. Sepkoski. 1993. Insect diversity in the fossil record: myth and reality. Science 261: 310–315.

Manchester, S. R. 1987. The fossil history of the Juglandaceae. Ann. Missouri Bot. Gard. 21: 1–137.

_____. 1989. Systematics and fossil history of the Ulmaceae. Pp. 221–251 *in* P. R. Crane and S. Blackmore (eds.), Evolution, Systematics, and Fossil History of the Hamamelidae, Vol. 2: 'Higher' Hamamelidae. Clarendon Press, Oxford.

Manning, W. E. 1959. *Alfaroa* and *Engelhardtia* in the New World. Bull. Torrey Bot. Club 86: 190–198.

Michener, C. D. and D. A. Grumaldi. 1988. A *Trionga* from late Cretaceous amber of New Jersey (Hymenoptera: Apidae: Melponinae). Amer. Mus. Novit. 2914: 1–10.

Muller, M. T. and D. L. Dilcher. 1991. Dichasia from the mid-Cretaceous of central North America. Amer. J. Bot., Abstracts 78: 120.

Niklas, K. J. 1981. Airflow patterns around some early seed plant ovules and cupules: implications concerning efficiency in wind pollination. Amer. J. Bot. 68: 635–650.

Norstog, K. J., D. W. Stevenson, and K. J. Niklas. 1986. The role of beetles in the pollination of *Zamia furfuracea* L. fil. (Zamiaceae). Biotropica 18: 300–306.

Pirozynski, K. A. and D. W. Malloch. 1975. The origin of land plants: a matter of mycotrophism. Biosystems 6: 153–164.

Proctor, M. C. and P. Yeo. 1972. The Pollination of Flowers. Taplinger Publ., New York.

Retallack, G. and D. L. Dilcher. 1981. Early angiosperm reproduction: *Prisca reynoldsii* gen. et sp. nov., from mid-Cretaceous coastal deposits in Kansas, U.S.A. Palaeontographica, Abt. B, Paläophytol. 179: 103–137.

Schlichting, C. D., A. G. Stephenson, L. E. Davis, and J. A. Winsor. 1987. Pollen competition and offspring variance. Evol. Trends Pl. 1: 35–39.

Scott, A. C. and T. N. Taylor. 1983. Plant/animal interactions during the Upper Carboniferous. Bot. Rev. (Lancaster) 49: 259–307.

Silvertown, J. 1987. Introduction to Plant Population Ecology, 2nd. ed. John Wiley and Sons, New York.

Stephenson, A. G. and R. I. Bertin. 1983. Male competition, female choice, and sexual selection in plants. Pp. 109–149 *in* L. A. Real (ed.), Pollination Biology. Academic Press, New York.

Stone, D. E. 1972. New Juglandaceae, III. A new perspective of the tropical members with winged fruits. Ann. Missouri Bot. Gard. 59: 297–321.

Taylor, D. W. and L. J. Hickey. 1990. An Aptian plant with attached leaves and flowers: implications for angiosperm origin. Science 247: 702–704.

Taylor, T. N. and E. L. Taylor. 1992. The Biology and Evolution of Fossil Plants. Prentice Hall, New Jersey.

_____ and M. S. Zavada. 1986. Developmental and functional aspects of fossil pollen. Pp. 165–177 *in* S. Blackmore and I. K. Ferguson (eds.), Pollen and Spores: Form and Function. Academic Press, London.

Tiffney, B. H. 1984. Seed size, dispersal syndromes, and the rise of the angiosperms: evidence and hypothesis. Ann. Missouri Bot. Gard. 71: 551–576.

Weberling, F. 1989. Morphology of Flowers and Inflorescences. Cambridge University Press, Cambridge.

Zavada, M. S. 1984. The relation between pollen exine sculpturing and self incompatibility mechanisms. Pl. Syst. Evol. 147: 63–78.

_____. 1990. A contribution to the study of pollen wall ultrastructure of orchid pollinia. Ann. Missouri Bot. Gard. 77: 785–801.

GENOTYPE-ENVIRONMENT INTERACTION, PARENTAL EFFECTS, AND THE EVOLUTION OF PLANT REPRODUCTIVE TRAITS[1]

Johanna Schmitt[2]

ABSTRACT. There is increasing evidence that the expression of genetic variation in natural plant populations may depend upon the environment. Such genotype × environment interaction (G × E) can have important implications for the evolution of plant reproductive traits. If there is G × E for a given trait, then the ability of researchers to detect genetic variation may depend upon the environment; moreover, the environment can affect the potential for selective agents to distinguish among genotypes, and consequently the potential for evolutionary response to phenotypic selection. G × E can also be viewed as genetic variation for phenotypic plasticity and is a necessary condition for reaction norms to evolve in response to natural selection. Here I discuss the implications of G × E, at both the parental and offspring levels, for the evolution of plant reproductive traits. In particular, I focus on genetic and environmental contributions to reproductive timing, using reaction norms to light availability in two populations of *Impatiens capensis* as an example. In this species, environmental sensitivity of axillary meristem allocation to early reproduction vs. branching may constitute a fundamental developmental constraint on the evolution of plasticity. I also review recent evidence for parental effects in plants, pointing out that maternal and paternal effects can be viewed as a form of phenotypic plasticity. There is increasing evidence for parental genotype × parental environment and parental genotype × offspring environment interactions for progeny traits in natural populations, suggesting the potential for such plasticity to evolve in response to selection.

Plant biosystematists and evolutionary biologists often have sought to deconfound the effects of genotype and environment on quantitative traits. For example, common garden experiments are typically used to control environmental variation in order to test for ecotypic differentiation; more recently, such experiments have also been used to examine microgeographic genetic differentiation within and between populations (Schemske, 1984; Linhart, 1988; Argyres and Schmitt, 1991). It is becoming increasingly apparent, however, that genotype and environment effects are not always independent in natural plant populations (Shaw, 1986; Garbutt and Bazzaz, 1987; Sultan, 1990; Schmitt et al., 1992; Schmitt, 1993; Bowers et al., 1992). If genotypes differ in plastic response to environmental variation, the expression of genetic variation and potential for response to natural selection may depend upon the environment. Moreover, there is now considerable evidence that maternal and paternal effects can influence the expression of offspring phenotype in plants (Schaal, 1984; Alexander and Wulff, 1985; Antonovics and Schmitt, 1986; Schmitt and Antonovics, 1986; Roach and Wulff, 1987; Biere, 1991a, 1991b; Lacey, 1991; Miao et al., 1991a, 1991b; Potvin and Charest, 1991; Richardson and Stephenson, 1992; Schmitt et al., 1992; Purrington 1993;

[1]A. Z. Argyres, M. Cheo, S. A. Dudley, J. Eccleston, D. W. Ehrhardt, S. E. Gamble, M. B. Hamilton, J. Karb, J. Niles, and R. D. Wulff have made major contributions to this research, which has been supported by NSF grants BSR-84000147, BSR-8906291, and BSR-9023389. I am grateful to S. A. Dudley, M. B. Hamilton, C. B. Purrington, C. D. Schlichting, and A. G. Stephenson for comments on the manuscript and to C. B. Purrington for the figures.

[2]Department of Ecology and Evolutionary Biology, Box G-W, Brown University, Providence, RI 02912, U.S.A.

Platenkamp and Shaw, 1993). Such parental effects can have an important impact on selection response (Kirkpatrick and Lande, 1989).

Here I discuss the implications of genotype × environment interaction (G × E), at both the parental and offspring levels, for the evolution of plant reproductive traits. In particular, I focus on genetic and environmental contributions to reproductive timing and argue that reproductive meristem allocation may represent a fundamental developmental constraint on the evolution of reproductive plasticity. I also point out that environmental maternal and paternal effects can be viewed as forms of phenotypic plasticity that can in themselves evolve in response to natural selection.

EVOLUTIONARY SIGNIFICANCE OF G × E

Environmental effects on phenotype can be characterized for a given genotype as a reaction norm, that is, the phenotypic expression of a given trait across a range of environments (Figure 1). Variation among genotypes in the magnitude or direction of this response will be detected as genotype × environment interaction in a two-way analysis of variance (Ayres and Thomas, 1990; Fry, 1992). If G × E exists within natural plant populations, it can have several important evolutionary consequences. G × E for a given trait can cause the expression of genetic variation for that trait (and consequently the ability of researchers and selective agents to discriminate among genotypes) to depend upon the environment (Sultan, 1987). For example, if genotypes differ in magnitude of plastic response to a particular environmental factor, considerable genetic variation may be apparent in certain environments, but masked in others (e.g., Figure 1b). Consequently, the genetic potential for evolutionary response to phenotypic natural selection will also depend upon the environment (Schlichting, 1989a). If reaction norms cross, the relative ranking of genotypes for a given trait will vary among environments (Figure 1c); if so, identical selective pressures on that trait could favor different genotypes in different environments. Moreover, it has been suggested that variation in reaction norms can lead to differences in the structure of genetic correlations within different environments (de Jong, 1990; Stearns, 1989; Stearns et al., 1991). If so, identical selective pressures in different environments will produce different evolutionary responses of correlated characters (Lande and Arnold, 1983).

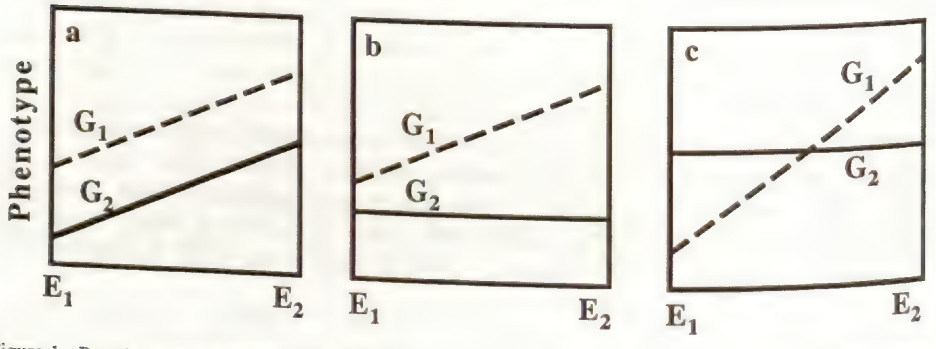


Figure 1. Reaction norms of two genotypes to two environments (E_1 and E_2). Each line connects the phenotypes expressed by a given genotype in the two environments. In (a), the effects of genotype and environment are additive; there is no G × E, and genotypes do not differ significantly in phenotypic plasticity. In (b), there is significant G × E; one genotype is very plastic, and the other does not respond to environment. Consequently, genetic variation is detectable in E_2 but not in E_1. In (c), the reaction norms cross, resulting in different rankings of genotypes in the two environments.

If significant G × E is detected within a plant population, it can be interpreted as genetic variation for phenotypic plasticity (Via and Lande, 1985). The idea of phenotypic plasticity as a distinct trait that can in itself evolve has been well recognized for some time (Bradshaw, 1965), but recently there has been a surge of interest in the evolution of reaction norms (Via and Lande, 1985; de Jong, 1990; van Tienderen, 1991; Stearns et al., 1991; Thompson, 1991; Gomulkiewicz and Kirkpatrick, 1992) and the evolutionary consequences of phenotypic plasticity in natural plant populations (Schlichting, 1986, 1989b; Sultan, 1987; Quinn, 1987; Kuiper and Kuiper, 1988; Levin, 1988; Bradshaw and Hardwick, 1989; van Tienderen, 1990). Phenotypic plasticity can only evolve toward an optimal reaction norm if there is within-population G × E (Via and Lande, 1985). The evolution of plasticity may be constrained if the phenotypic expression of a given trait displays strong genetic correlation across environments (Via and Lande, 1985; Gomulkiewicz and Kirkpatrick, 1992), developmental constraints (van Tienderen, 1990), or physiological costs of plasticity (van Tienderen, 1991; Gomulkiewicz and Kirkpatrick, 1992). To understand how phenotypes evolve in variable environments, it is therefore important to investigate variation in reaction norms within and between natural populations. Only recently, however, have such studies been performed in wild plants (Shaw, 1986; Garbutt and Bazzaz, 1987; Sultan, 1990; Mazer and Schick, 1991a, 1991b; Bowers et al., 1992; Schmitt et al., 1992; Schmitt, 1993).

REACTION NORMS OF REPRODUCTIVE PHENOLOGY AND MERISTEM ALLOCATION

Reproductive phenology is an ecologically important trait that may often be subject to strong selection (Schmitt, 1983; Schemske, 1984; Rathcke and Lacey, 1985). Genetic variation in reproductive phenology within and between populations has often been noted (Schemske, 1984, Rathcke and Lacey, 1985; Quinn, 1987), but phenological traits also display considerable environmental plasticity (Rathcke and Lacey, 1985; Simpson et al., 1985; Quinn, 1987; Schmitt et al., 1987a). In many plant species, flowering is triggered by environmental cues or resource availability. It is therefore of interest to ask whether genetic variation exists between or within natural populations for plasticity of flowering phenology.

The timing of reproduction is also of interest because it is a critical component of a plant's life history. It has been suggested that allocation of resources to early reproduction may result in a tradeoff with survival, growth, and later fecundity (Reznick, 1985; Bell and Koufopanou, 1986; Stearns, 1989; Geber, 1990; Dorn and Mitchell-Olds, 1991). In plants, such tradeoffs also may be generated because the commitment of a meristem to reproductive structures precludes vegetative growth from that meristem (Watson, 1984; Smith, 1984; Geber, 1990). For example, Geber (1990) observed genetic variation in commitment of axillary meristems to reproduction vs. branching and vegetative growth in the annual species *Polygonum arenastrum*. This variation resulted in correlated genetic variation in developmentally linked life history traits such as age of first reproduction and early and late fecundity and growth; there were significant tradeoffs between early reproduction and later growth and fecundity. If such patterns of meristem commitment are responsive to environmental cues, the potential exists for integrated plastic response (Schlichting, 1989b) of a whole suite of developmentally linked traits (Schmitt, 1993).

Impatiens capensis Meerb. (Balsaminaceae), touch-me-not or jewelweed, provides an ideal system for examining plasticity of flowering phenology and reproductive meristem allocation in response to resource availability. Jewelweeds are common annuals throughout northeastern North America; they occur over an unusually broad range of light environments, ranging from full sun in wet disturbed areas and stream banks to densely shaded forest

understory (Waller, 1980; Simpson et al., 1985). Light availability can vary on a fine spatial scale within populations (Stewart and Schoen, 1987), and smaller plants are often shaded by their taller neighbors (Cid-Benevento and Schaal, 1986). *Impatiens* plants produce both obligately self-fertilizing cleistogamous (CL) flowers and protandrous, animal-pollinated, highly outcrossing chasmogamous (CH) flowers. As in *Polygonum* (Geber, 1990), the first CL flowers in *I. capensis* are produced at axillary meristems on the main stem, precluding the formation of a primary branch from that meristem (Schmitt et al., 1987a; Schmitt and Hamilton, in prep.). Jewelweed flowering phenology and percentage of CH flower production are extremely plastic in response to environmental variation (Waller, 1980; Simpson et al., 1985; Schmitt et al., 1987a). However, *Impatiens* can also display dramatic genetic differentiation within and between populations for flowering time and other life history and morphological traits (Schemske, 1984; Simpson et al., 1985; Argyres and Schmitt, 1991), some of which is attributable to local adaptation (Schemske, 1984; Schmitt and Gamble, 1990; Argyres and Schmitt, 1991).

I examined reaction norms of reproductive and life history traits to light availability within and between two neighboring natural *I. capensis* populations from contrasting light environments (Schmitt, 1993). The two populations were approximately 1 km apart at Brown University's Haffenreffer Reserve (Bristol, RI). The "sun" population was a dense stand of jewelweed growing at high density (2000–3000 seedlings/m^2) in full sun on a sewage outflow (Schmitt et al., 1987a, 1987b; Schmitt and Ehrhardt, 1990). In this population there was substantial density-dependent mortality, but many plants remained flowering until the first hard frost in early October (Schmitt et al., 1987a, 1987b). The "woods" site was in the understory of an oak-hickory forest; seedling densities were an order of magnitude lower (200–300/m^2) and pathogen-induced mortality occurred much earlier in the season, with no survival beyond mid-August (Schmitt and Gamble, 1990).

Seedlings were collected randomly from each population and grown to maturity in a greenhouse in two light environments: full light and neutral shade to 18% of full light (Schmitt, 1993). Selfed full-sib seed families were collected from plants from both populations grown in both parental light environments. To characterize family reaction norms to light availability (Schmitt, 1993), and to examine genetic correlations among traits within each environment (Schmitt and Hamilton, in prep.), seedlings from each family were divided into the same two light treatments in March of the following year and scored for a variety of characters; here I will focus on date of first reproduction, meristem allocation to early CL reproduction (May), cumulative meristem allocation to CH and CL reproduction (August), and cumulative number of branches (August). The maternal light environment had little impact on progeny reaction norms and will not be discussed further here (see Schmitt, 1993 for details).

The two populations differed dramatically in plastic response of reproductive timing to light availability (Figure 2). Plants from the woods population displayed fixed patterns of meristem allocation; regardless of light availability, they flowered early at the lower nodes, resulting in high early reproduction and little branching (Figure 2). Thus, in this population a fixed developmental pattern precluded plastic response to light for a whole suite of traits. In contrast, plants from the sun population reproduced later overall and allocated more axillary meristems to branches rather than flowers, but the pattern of meristem allocation was very sensitive to light. Exposure to shade caused sun population plants to allocate more axillary meristems to early reproduction, resulting in earlier flowering and reduced branching relative to siblings grown in full sun (Figure 2). Thus, in this population plasticity of meristem allocation resulted in integrated plastic response for a wide range of characters. Differences between the two populations in light sensitivity of meristem allocation produced concurrent differences in reaction norms for a suite of developmentally linked traits.

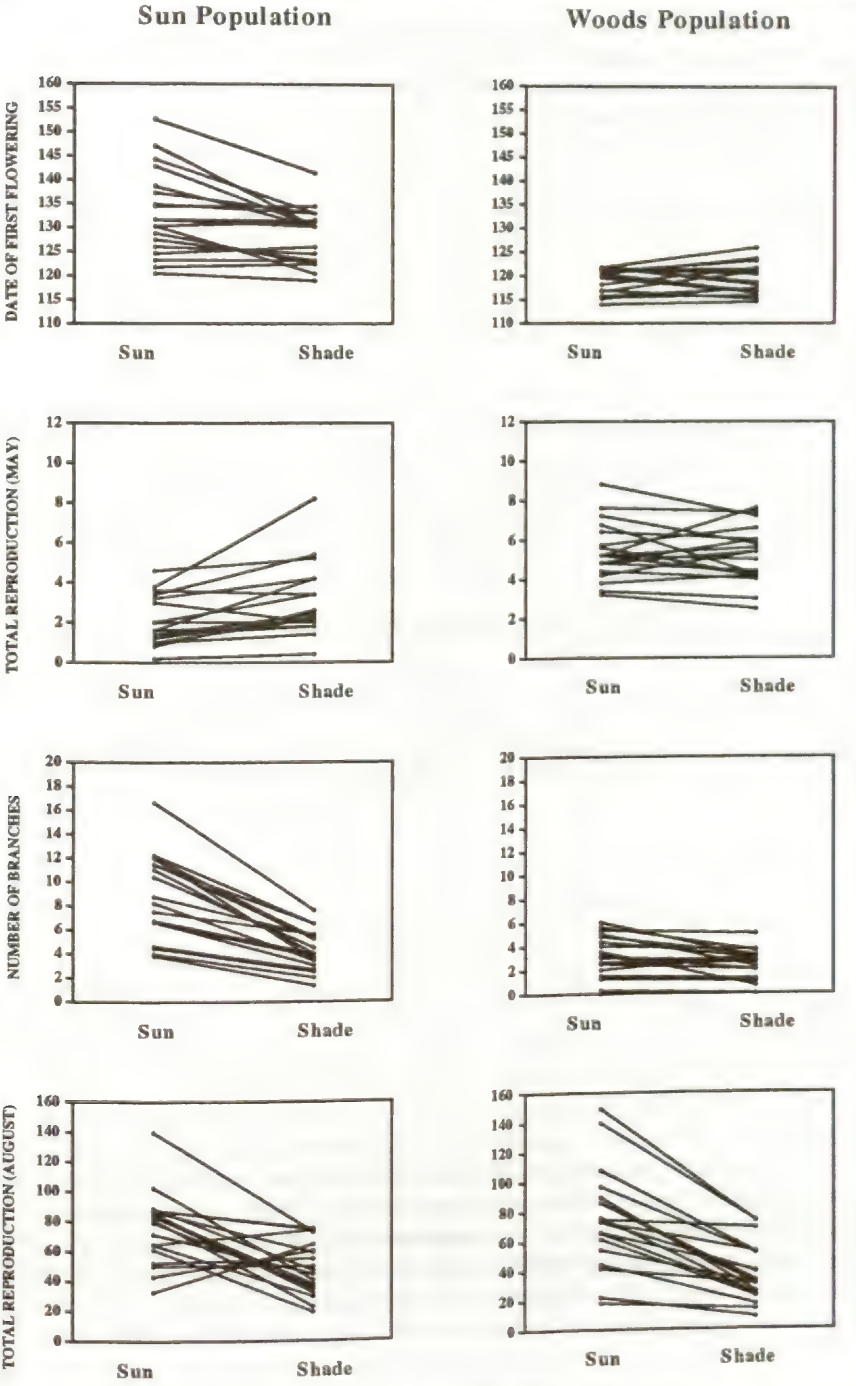


Figure 2. Reaction norms of meristem allocation and reproductive traits to light availability in *Impatiens capensis* (from Schmitt, 1993). Mixed model analysis of covariance revealed significant G × E within the sun population for date of first reproduction ($p < 0.025$), early (May) reproduction ($p < 0.005$), branch number ($p < 0.05$), and total (August) reproduction ($p < 0.001$), indicating genetic variation for plasticity. In contrast, in the woods population, there was no significant G × E for any of these traits.

The insensitivity of meristem allocation to light in the woods population constitutes a strong developmental constraint on the evolution of plasticity (van Tienderen, 1990). In contrast, there was substantial genetic variation in plastic response to light within the sun population, indicated by significant G × E effects for date of reproduction, early reproduction, number of branches, and cumulative reproduction (Figure 2). The observed variation in reaction norms appears to reflect underlying genetic variation in sensitivity of meristem allocation to light availability. This genetic variation provides a potential substrate for natural selection on reaction norms if plants in the sun population experience variable light environments. However, all meristem allocation traits except total reproduction displayed significant positive correlations across environments (Table 1), which could constrain the rate of evolution toward an optimal reaction norm in a heterogeneous light environment (Via and Lande, 1985).

TABLE 1. Genetic correlations of meristem allocation traits and total reproduction within and between light environments for the sun and woods populations. Within-environment family mean correlations, from Schmitt and Hamilton (unpubl.), were calculated using residuals from an analysis of covariance to remove maternal effects due to seed weight, emergence date, and maternal environment. Correlations above the diagonal are for the shade treatment; those below the diagonal are for the full light treatment. Correlations along the diagonal are across-environment genetic correlations from Schmitt (1992) calculated from two-way analysis of variance, as described by Fry (1992). For the woods population, these estimates were all > 1.

	Fruiting Date	Early Reproduction	Branches	Total Reproduction
Woods				
fruiting date	1.00***	-0.78***	0.02	-0.39
early reproduction	-0.71***	1.00*	0.16	0.52*
branches	0.37	-0.31	1.00***	0.78***
Total Reproduction	-0.14	0.22	0.62***	1.00**
Sun				
fruiting date	0.77**	-0.79***	0.53*	0.45+
early reproduction	-0.75***	0.77**	-0.61**	-0.51*
branches	0.56*	-0.72***	0.82***	0.87***
Total Reproduction	-0.08	-0.12	0.04	0.15

$^{+}p < 0.07$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

The pattern of broad-sense genetic correlations among traits also differed dramatically between populations and depended upon the light environment (Schmitt and Hamilton, unpubl.). In the woods population, genetic correlations between early reproduction and branching were relatively small and not significant (Table 1). There was a significant positive genetic correlation between early and late reproduction in the shade treatment, and a nonsignificant positive correlation in full light. Thus, in this population there is no evidence for genetic variation in meristem allocation to early reproduction vs. branches, and no evidence for tradeoffs among genotypes in allocation to early vs. late reproduction. Such tradeoffs may exist in this population, but if so they are probably masked by positive genetic correlations due to variation in overall growth ability (van Noordwijk and de Jong, 1986; Stearns, 1989; Houle, 1991). In contrast, in the sun population, there were strong negative genetic correlations between early reproduction and branching in both light environments (Table 1), indicating substantial genetic variation in pattern of axillary meristem allocation (Schmitt and Hamilton, unpubl.). In this population, selection for earlier reproduction should produce

reduced branching as a correlated response. There was also a strong negative genetic correlation between early and total reproduction in the shade treatment, indicating a cost of early reproduction. This result contrasts dramatically with the positive correlation observed for the woods population in the same environment. The tradeoff was not significant in the full light environment, although it may have been masked by genetic variation in flowering time; later-flowering plants had not yet completed the production of reproductive meristems (Schmitt and Hamilton, unpubl.), and a stronger tradeoff might have been apparent if the experiment had run longer (Geber, 1990).

These results are important in light of current interest in the question of whether genetic correlations among traits differ among populations (Venable and Búrquez, 1991) or depend upon the environment (Schlichting, 1986; de Jong, 1990; Stearns, 1989; Stearns et al., 1991) and have critical implications for the potential for evolutionary response to natural selection on correlated characters within the two populations (Schmitt and Hamilton, unpubl.). In the woods population, there appears to be little genetic variation in allocation of axillary meristems; response to selection for increased early reproduction would not be at the expense of branching or later reproduction. In contrast, in the sun population, selection for early reproduction would result in reduced branching and, at least in low light conditions, decreased total reproduction if plants survive for several months. Thus, the two populations would follow different evolutionary trajectories in response to identical selection in identical environments. Moreover, the observation that genetic correlations vary with light availability suggests that within a population, the response to correlated selection can depend upon the light environment.

What selective pressures might lead to such dramatic genetic divergence of neighboring populations? Schemske (1984) demonstrated that within-population genetic differentiation in flowering times in *Impatiens pallida* between a forest understory site and a site on the edge of a road could be attributed to selection for earlier reproduction due to earlier mortality in the woods. The woods population of *I. capensis* described here has probably undergone similar strong selection for early flowering; mortality occurs early in the season, and no plants survive beyond mid-August (Schmitt and Gamble, 1990; Dudley and Schmitt, unpubl.). In contrast, in the sun population, large plants persist and flower until October (Schmitt et al., 1987a, 1987b). Given a short growing season, the early reproduction of plants from the woods would result in higher relative fitness. If the experiment described here had been terminated after two months, early-flowering plants from the woods would have had a fitness advantage in both environments. It therefore seems likely that early mortality in the woods population has selected for fixed patterns of meristem allocation to early reproduction, altering the pattern of genetic correlations and precluding phenotypic plasticity.

But why should meristem allocation display plastic response to light availability in the sun population? Such a response might be adaptive if light availability provides predictive information about a plant's expected lifespan. Since light is a critical resource, light availability may both predict and influence longevity. The level of irradiance experienced by a plant may depend strongly upon neighbor density and relative position in the size hierarchy. For example, in *Ambrosia trifida*, the average light received by leaves decreases with plant density and is also much more variable in high-density stands, presumably because smaller plants are shaded by their neighbors (Jurik, 1991). In *Impatiens pallida*, smaller plants receive significantly lower irradiance (Cid-Benvenuto and Schaal, 1986).

In the sun population of *I. capensis* described here, both density and plant size can strongly affect the risk of mortality. Experimental density manipulations have demonstrated that the risk of early mortality increases significantly with density (Schmitt et al., 1987b). Moreover, this risk is size-dependent; smaller plants have slower relative growth rates and

shorter lifespans (Schmitt et al., 1987b). If light availability is a good indicator of density and position in the size hierarchy, increased allocation of axillary meristems to early reproduction in response to shading may be adaptive. On the other hand, plants in low density stands or at the top of the canopy have longer expected lifespans and may maximize fitness by allocating meristems to branches that will increase the potential for growth and later reproduction (Smith, 1984; Geber, 1990; Schmitt and Hamilton, unpubl.). In fact, smaller plants in the sun population display accelerated reproduction at axillary meristems, and early reproduction decreases later growth; CL flowering is significantly earlier at higher density (Schmitt et al., 1987a). The results of the reaction norm experiment (Schmitt, 1993) suggest that sensitivity to light availability may be the proximate mechanism for the plastic responses to density observed in the field. In this population, sensitivity of axillary meristem allocation to light availability may be a means of adaptation to fine-scale heterogeneity in the light environment due to competitive interactions.

Such adaptive hypotheses can only be tested by direct observations of selection in natural populations. To demonstrate that sensitivity of meristem allocation is adaptive in the sun population, it will be necessary to demonstrate both that early reproduction confers a fitness advantage on plants shaded by neighbors, and that delayed reproduction is advantageous for plants experiencing high levels of irradiance (Schmitt and Wulff, 1993). Phenotypic selection analysis, coupled with experimental manipulation (Lande and Arnold, 1983; Mitchell-Olds and Shaw, 1987; Wade and Kalisz, 1990), provides a powerful tool for testing such predictions (Schmitt and Wulff, 1993).

ENVIRONMENTAL PARENTAL EFFECTS AS REACTION NORMS

Genotype × environment interaction may also play an important role in the evolution of parental effects. The ecological and evolutionary impact of maternal and paternal effects in plants has recently received considerable attention (Schaal, 1984; Alexander and Wulff, 1985; Antonovics and Schmitt, 1986; Schmitt and Antonovics, 1986; Mazer, 1987; Roach and Wulff, 1987; Stratton, 1989; Lacey, 1991; Miao et al., 1991a, 1991b; Biere, 1991a, 1991b; Potvin and Charest, 1991; Richardson and Stephenson, 1992; Schmitt et al., 1992; Purrington, 1993; Platenkamp and Shaw, 1993). Roach and Wulff (1987) distinguish three mechanisms whereby the maternal parent can contribute to offspring phenotype in a non-Mendelian manner: cytoplasmic genetic, endosperm nuclear, and maternal phenotypic; these mechanisms can also produce paternal effects. Cytoplasmic genetic effects result from non-Mendelian inheritance of mitochondria and plastids. Transmission of mitochondrial DNA is largely maternal in plants; plastid DNA is often maternally transmitted in angiosperms, but can also be inherited biparentally or paternally (Corriveau and Coleman, 1988). Endosperm nuclear parental effects in angiosperms result from double fertilization, which produces unequal dosages of maternal and paternal genomes in the endosperm. Endosperm parental effects may have a particular impact on offspring juvenile traits, since the endosperm is important for germination and as a nutrient source during seedling development. Maternal phenotypic effects are particularly interesting because they include influences of both maternal genotype and maternal environment (Roach and Wulff, 1987). The seed coat and surrounding ovary structures are entirely maternal in origin, and often play important roles in dormancy, germination, resistance of seeds to predators or pathogens, and dispersal. Maternal genotype and environment can also influence progeny phenotype via resource provisioning or regulatory influences during seed development (Potvin and Charest, 1991). Similarly, paternal genotype and environment may influence pollen grain performance (Young and Stanton, 1990; Quesada et al., 1991; Stephenson, 1992), and these effects could potentially be expressed in the progeny.

There is mounting evidence that both maternal genotype and environmental maternal effects can influence progeny phenotype in wild plant species. This evidence comes from both reciprocal crosses and environmental studies (Roach and Wulff, 1987). Since reciprocal full-sibs have the same parental nuclear genetic contribution, phenotypic differences between them are attributed to maternal and paternal effects (Antonovics and Schmitt, 1986; Mazer, 1987; Weis et al., 1987; Biere, 1991a, 1991b; Morse and Schmitt, 1991); greater variance among maternal families than paternal families is taken as evidence for maternal effects. Mating designs such as the diallel cross and the North Carolina Type II factorial cross permit variance in offspring phenotype to be partitioned into nuclear and reciprocal general and specific effects; reciprocal general effects can be decomposed into maternal and paternal components (Cockerham and Weir, 1977; Antonovics and Schmitt, 1986; Morse and Schmitt, 1991; Biere, 1991a, 1991b). Reciprocal effects can also be partitioned into genetic and environmental components by using replicate crosses (Antonovics and Schmitt, 1986; Biere, 1991a; Lacey, 1991). Considerable evidence of environmental parental effects in wild plant species has also been detected by experimentally manipulating the parental environment and examining the effect on offspring phenotype (Alexander and Wulff, 1985; Wulff, 1986; Roach and Wulff, 1987; Stratton, 1989; Aarssen and Burton, 1990; Miao et al., 1991a, 1991b; Potvin and Charest, 1991; Schmitt et al., 1992; Lacey, 1991; Wulff and Bazzaz, 1992; Purrington, 1993; Platenkamp and Shaw, 1993). The expression of parental effects may depend upon the offspring environment (Alexander and Wulff, 1985; Parrish and Bazzaz, 1985; Stratton, 1989; Miao et al., 1991a, 1991b; Potvin and Charest, 1991; Schmitt et al., 1992; Wulff and Bazzaz, 1992). For example, endosperm dosage effects or seed provisioning may have a greater impact on seedling growth and physiology if the progeny are subjected to low resource availability or environmental stress.

Parental effects in natural populations are potentially important because they may influence the level at which natural selection discriminates among genotypes and the rate and nature of evolutionary response to natural selection. If juvenile phenotypes are largely maternally determined, then natural selection will not discriminate among genotypes within a sibship, but may act at the maternal genotype level (Schmitt and Antonovics, 1986; Biere, 1991a, 1991b; Platenkamp and Shaw, 1993). Environmental maternal effects may mask genetic variation and thereby inhibit selection response (Stratton, 1989). Maternal inheritance can cause time lags in evolutionary response to selection; maternal selection, in which maternal traits influence offspring fitness, can in some cases lead to maladaptive evolution away from a fitness peak (Kirkpatrick and Lande, 1989).

Environmental parental effects also have practical implications for the design and interpretation of biosystematic studies. If such effects are important in natural populations, then common garden or reciprocal transplant experiments may detect apparent genetic differentiation that is actually due to environmental differences between parental source populations (Nelson et al., 1970; Baskin and Baskin, 1973; Quinn and Colosi, 1977; Roach and Wulff, 1987). This problem is especially serious if environmental parental effects persist for more than one generation, as suggested by several recent studies (Alexander and Wulff, 1985; Miao et al., 1991b). Little is known, however, about how naturally occurring environmental heterogeneity within or between wild plant populations affects progeny phenotype. In *Plantago lanceolata*, clonal replicates of wild collected genotypes planted back into natural source populations in a mowed lawn and an abandoned hayfield in a reciprocal transplant design displayed strong parental environmental effects on seed traits (Schmitt et al., 1992). More such field studies are needed to characterize the potential impact of parental effects generated by environmental variation within and between natural plant populations.

Recently it has been suggested that parental environmental effects can be viewed as a form of plasticity that can in itself evolve (Schmitt et al., 1992; Lacey, 1991; Platenkamp and Shaw, 1993). If there is a strong correlation between parental and offspring environments, then parental effects enhancing offspring fitness in the parental environment will be advantageous (Roach and Wulff, 1987). For example, if selection for large seeds is stronger in shaded environments, parent plants that produce larger seeds when exposed to shade may have a fitness advantage. For such adaptive plasticity to evolve, there must be variation in maternal reaction norms of progeny phenotype to maternal environments within natural populations, detected as a significant maternal genotype × maternal environment interaction effect. Such interactions have recently been observed in several wild plant species (Alexander and Wulff, 1985; Schmitt et al., 1992; Lacey, 1991; Wulff and Bazzaz, 1992; Platenkamp and Shaw, 1993). Thus, the potential may exist for the evolution of plastic response of maternally determined offspring traits to maternal environments. Moreover, if such G × E exists, the ability of natural selection to discriminate among maternal genotypes may depend upon the parental environment (Schmitt et al., 1992).

It is also of interest to examine parental reaction norms of progeny phenotypes to progeny environments. Sensitivity of germination to environmental cues such as light, temperature, or nutrients is often thought to be adaptive, because it will increase the probability that seedlings will encounter favorable conditions for establishment (Fenner, 1985). The potential for such adaptive plasticity to evolve in response to selection may depend strongly upon the extent of variation in reaction norms among maternal genotypes, since germination behavior is influenced by maternal effects acting through the seed coat and endosperm. In some cases, plastic response to parental environments may depend upon the offspring environment, and vice versa. For example, in certain species, exposure of seeds to chlorophyll-filtered light during development will induce a phytochrome-mediated light requirement for germination; among species, the ability to germinate in the dark is inversely correlated with the chlorophyll content of the structures surrounding the developing seed (Cresswell and Grime, 1981). If maternal genotypes differ in the chlorophyll content of these investing structures within a species, it may lead to variation in reaction norms to both parental and offspring light environments. Genotypes with high levels of chlorophyll surrounding the seeds will have strong light requirements for germination regardless of the parental environment. However, for genotypes with little chlorophyll in the investing structures, the phytochrome photoequilibrium in the developing seed will be affected by the parental light environment; exposure of the maternal parent to foliage shade may induce a light requirement for germination in the progeny.

Several recent studies provide evidence for maternal genotype × offspring environment interaction in natural populations (Alexander and Wulff, 1985; Schmitt and Antonovics, 1986; Schmitt et al., 1992; Wulff and Bazzaz, 1992; also see Venable et al., 1987; Venable and Búrquez, 1989), suggesting that the potential may exist for natural selection to act upon maternal reaction norms at this level. For example, in *Plantago lanceolata*, maternal genotypes differed significantly in plastic response of progeny germination rate to light or dark (Schmitt et al., 1992). As a result, large differences among maternal genotypes in germination rate could be detected for progeny exposed to a 14-hr photoperiod; in contrast, seeds exposed to darkness displayed significant parental environmental effects but no detectable variation among maternal genotypes. These results suggest that natural selection on germination rate might discriminate among maternal genotypes for seeds on the surface of the soil, but not for buried seeds. Thus, variation in maternal reaction norms to progeny environments can have an impact on the potential for response to selection, as well as genetic population structure. If maternal genotypes differ in germination requirements, it may lead to differential response of maternal families to spatial and/or temporal environmental heterogeneity, and thus to genetic

variation of seedlings in time and space. For example, in a natural population of *Anthoxanthum odoratum*, maternal genotypes vary in relative emergence probability over a spatial scale of a few meters (Schmitt and Antonovics, 1986). Such G × E may help to maintain genetic variation for seed and seedling traits within natural populations (Schmitt and Antonovics, 1986; Platenkamp and Shaw, 1993).

DIRECTIONS FOR FUTURE RESEARCH

The evidence to date indicates that G × E at both parental and offspring levels may be relatively common in natural plant populations and may have an important impact on the evolution of reproductive traits. To understand the potential for plastic response to a particular ecological factor to evolve, studies are needed in which genetic variation for reaction norms to that factor is characterized within populations. At the same time, within the same populations, it is important to characterize the scale and pattern of environmental heterogeneity for the same ecological factor in space and time and to measure the strength and direction of natural selection on the traits of interest over the range of environments experienced. In particular, to demonstrate that a particular plastic response is adaptive, it is necessary to show that the phenotype induced by a given environment results in higher fitness in that environment and is selected against in environments in which it is not induced (Schmitt and Wulff, 1993). This can be done using phenotypic selection analysis (Lande and Arnold, 1983; Mitchell-Olds and Shaw, 1987; Wade and Kalisz, 1990), as well as by experimental manipulation of phenotypes to compare the relative fitness of "appropriate" and "inappropriate" morphologies within each environment of interest (Schmitt and Wulff, 1993). Combined genetic and selection studies are particularly needed to understand the evolution of maternal and paternal effects in plants and the level at which natural selection acts.

It is also important to investigate possible genetic, physiological, and developmental constraints on the evolution of plasticity. To address these questions, attention to the developmental and physiological mechanisms underlying reaction norms is needed. Information is needed not only about the strength and sign of genetic correlations of trait expression across environments, and between traits within environments, but also about how functional relationships determine the nature of these genetic correlations (Stearns, 1989; Stearns et al., 1991; Houle, 1991). Other critical questions are whether there is a cost of plastic response (van Tienderen, 1991), and how fixed developmental programs may constrain the expression of optimal plastic response (van Tienderen, 1990).

Another important issue is the potential impact of inbreeding on reaction norms of phenotype, performance, and fitness. It has been suggested that developmental homeostasis will increase with heterozygosity, and consequently that outcrossed or heterozygous genotypes will be less variable over a range of environments (Falconer, 1989; Gillespie and Turelli, 1989). This hypothesis implies that it is advantageous to maintain a constant phenotype across environments. On the other hand, such homeostasis may require adaptive plasticity of other traits. Relatively little is known about how inbreeding affects the expression of morphological (but see Willis, 1991), life history, or physiological traits in wild plant species. Even less is known about how individual inbred and outcrossed genotypes from natural plant populations differ in reaction norms of such traits to variable environments (but see Schlichting and Levin, 1986; Jinks and Pooni, 1988, for discussion of data from cultivated species). There is, however, increasing evidence that the relative fitness of inbred and outcrossed individuals may depend upon the environment (Dudash, 1990; Schmitt and Ehrhardt, 1990; Schmitt and Gamble, 1990), and it will be of interest to ask how these fitness responses are related to the expression of phenotype across environments.

Plants provide attractive systems for integrating functional and evolutionary approaches to the study of reaction norms. For example, responses to light, water, and nutrient availability have often been investigated from a physiological perspective, but only recently has this functional approach been applied in an evolutionary framework (Sultan, 1990; Dudley, 1991). Phytochrome-mediated developmental responses to light quality are another promising area for the study of adaptive plasticity, since they are well studied at the molecular genetic and physiological levels and have important ecological consequences, both for seed germination and for morphological responses of plants to foliage shade and crowding (Casal and Smith, 1989; Schmitt and Wulff, 1993). The results presented here indicate that environmental sensitivity of meristem allocation may also be a valuable system for investigating the fitness consequences and functional basis of phenotypic plasticity. The opportunity now exists to integrate genetic, physiological, and ecological approaches to the study of reaction norms; such integration may provide important insights about the evolution of plasticity in natural plant populations.

LITERATURE CITED

Aarssen, L. W. and S. M. Burton. 1990. Maternal effects at four levels in *Senecio vulgaris* (Asteraceae) grown on a soil nutrient gradient. Amer. J. Bot. 77: 1231–1240.

Alexander, H. M. and R. D. Wulff. 1985. Experimental ecological genetics in *Plantago*. X. The effects of maternal temperature on seed and seedling characters in *P. lanceolata*. J. Ecol. 73: 271–282.

Antonovics, J. and J. Schmitt. 1986. Paternal and maternal effects on propagule size in *Anthoxanthum odoratum*. Oecologia 69: 277–282.

Argyres, A. Z. and J. Schmitt. 1991. Microgeographic genetic structure of morphological and life history traits in a natural population of *Impatiens capensis*. Evolution 45: 178–189.

Ayres, M. P. and D. L. Thomas. 1990. Alternative formulations of the mixed-model ANOVA applied to quantitative genetics. Evolution 44: 221–226.

Baskin, J. M. and C. C. Baskin. 1973. Plant population differences in dormancy and germination characteristics of seeds: heredity or environment? Amer. Midl. Naturalist 90: 493–498.

Bell, G. and V. Koufopanou. 1986. The cost of reproduction. Oxford Surv. Evol. Biol. 3: 83–131.

Biere, A. 1991a. Parental effects in *Lychnis flos-cuculi*. I: Seed size, germination, and seedling performance in a controlled environment. J. Evol. Biol. 3: 447–465.

_____. 1991b. Parental effects in *Lychnis flos-cuculi*. II: Selection on time of emergence and seedling performance in the field. J. Evol. Biol. 3: 467–486.

Bowers, M. D., S. K. Collinge, S. E. Gamble, and J. Schmitt. 1992. Effects of genotype, habitat, and seasonal variation on iridoid glycoside content of *Plantago lanceolata* (Plantaginaceae) and the implications for insect herbivores. Oecologia 91: 201–207.

Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances Genet. 13: 115–155.

_____ and K. Hardwick. 1989. Evolution and stress-genotypic and phenotypic components. Biol. J. Linn. Soc. 37: 137–155.

Casal, J. J. and H. Smith 1989. The function, action, and adaptive significance of phytochrome in light grown plants. Pl. Cell Environm. 12: 855–862.

Cid-Benevento, C. R. and B. A. Schaal. 1986. Variation in population growth rate in the woodland annual *Impatiens pallida* (Balsaminaceae). Amer. J. Bot. 73: 1031–1042.

Cockerham, C. C. and B. S. Weir. 1977. Quadratic analyses of reciprocal crosses. Biometrics 33: 187–203.

Corriveau, J. L. and A. W. Coleman. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Amer. J. Bot. 75: 1443–1458.

Cresswell, E. G. and J. P. Grime. 1981. Induction of a light requirement during seed development and its ecological consequences. Nature 291: 583–585.

Dorn, L. A. and T. Mitchell-Olds. 1991. Genetics of *Brassica campestris*. I. Genetic constraints on evolution of life-history characters. Evolution 45: 371–379.

Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. Evolution 44: 1129–1139.

Dudley, S. A. 1991. Local population evolution of physiological traits in *Cakile edentula* var. *lacustris* in response to differing water availability. Ph. D. thesis, University of Chicago.

Falconer, D. S. 1989. Introduction to Quantitative Genetics. John Wiley and Sons, New York.

Fenner, M. 1985. Seed Ecology. Chapman and Hall, New York.

Fry, J. D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. Evolution 46: 540–550.

Garbutt, K. and F. A. Bazzaz. 1987. Population niche structure: Differential response of *Abutilon theophrasti* progeny to resource gradients. Oecologia 72: 291–296.

Geber, M. A. 1990. The cost of meristem limitation in *Polygonum arenastrum*: negative correlations between fecundity and growth. Evolution 44: 799–818.

Gillespie, J. H. and M. Turelli. 1989. Genotype-environment interaction and the maintenance of polygenic variation. Genetics 121: 129–138.

Gomulkiewicz, R. and M. Kirkpatrick. 1992. Quantitative genetics and the evolution of reaction norms. Evolution 46: 390–411.

Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution 45: 630–648.

Jinks, J. L. and H. S. Pooni. 1988. Pp. 505–522 *in* B. S. Weir, E. J. Eisen, M. M. Goodman, and G. Namkoong, (eds.), Proceedings of the Second International Congress on Quantitative Genetics. Sinauer, Sunderland, Massachussetts.

Jong, G. de. 1990. Quantitative genetics of reaction norms. J. Evol. Biol. 3: 447–468.

Jurik, T. W. 1991. Population distributions of plant size and light environment of giant ragweed (*Ambrosia trifida* L.) at three densities. Oecologia 87: 539–550.

Kirkpatrick, M. and R. Lande. 1989. The evolution of maternal characters. Evolution 43: 485–503.

Kuiper, D. and P. J. C. Kuiper. 1988. Phenotypic plasticity in a physiological perspective. Acta Oecol., Oecol. Pl. 9: 43–59.

Lacey, E. L. 1991. Parental effects on life history traits in plants. Pp. 735–744 *in* E. C. Dudley (ed.), The Unity of Evolutionary Biology: Proceedings, IV International Congress of Systematic and Evolutionary Biology, Vol. 1. Dioscorides Press, Portland, Oregon.

Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37: 1210–1226.

Levin, D. A. 1988. Plasticity, canalization, and evolutionary stasis in plants. Pp. 35–45 *in* A. J. Davy, M. J. Hutchings, and A. R. Watkinson (eds.), Plant Population Biology. Blackwell Scientific Publications, Oxford.

Linhart, Y. B. 1988. Intrapopulation differentiation in annual plants. III. The contrasting effects of intra- and interspecific competition. Evolution 42: 1047–1064.

Mazer, S. J. 1987. Parental effects on seed development and seed yield in *Raphanus raphanistrum*: implications for natural and sexual selection. Evolution 41: 355–371.

_____ and C. T. Schick. 1991a. Constancy of population parameters for life history and floral traits in *Raphanus sativus* L. I. Norms of reaction and the nature of genotype by environment interactions. Heredity 67: 143–156.

_____ and _____. 1991b. Constancy of population parameters of life history and floral traits in *Raphanus sativus* L. II. Effects of planting density on phenotype and heritability estimates. Evolution 45: 1888–1907.

Miao, S. L., F. A. Bazzaz, and R. B. Primack. 1991a. Effects of maternal nutrient pulse on reproduction of two colonizing *Plantago* species. Ecology 72: 586–596.

_____, _____, and _____. 1991b. Persistence of maternal nutrient effects in *Plantago major*; the third generation. Ecology 72: 1634–1642.

Mitchell-Olds, T. and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41: 1149–1161.

Morse, D. H. and J. Schmitt. 1991. Maternal and paternal effects on follicle production in the milkweed *Asclepias syriaca* (Asclepiadaceae). Amer. J. Bot. 78: 1304–1309.

Nelson, J. R., G. A. Harris, and C. J. Goebel. 1970. Genetic vs. environmentally induced variation in medusahead (*Tainatherium asperum* [Simonkai] Nevski). Ecology 51: 526–529.

Noordwijk, A. J. van, and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Amer. Naturalist 128: 137–142.

Parrish, J. D. and F. A. Bazzaz. 1985. Nutrient content of *Abutilon theophrasti* seeds and the competitive ability of the resulting plants. Oecologia 65: 247–251.

Platenkamp, G. A. J. and R. G. Shaw. 1993. Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. Evolution 47: 540–555.

Potvin, C. and C. Charest. 1991. Maternal effects of temperature on metabolism in the C_4 weed *Echinochloa crusgalli*. Ecology 72: 1973–1979.

Purrington, C. B. 1993. Parental effects on progeny sex ratio, emergence, and flowering in *Silene latifolia* (Caryophyllaceae). J. Ecol. 81: 807–811.

Quesada, M., J. A. Winsor, and A. G. Stephenson. 1991. Effects of genotype on pollen performance in *Cucurbita pepo*. Sexual Pl. Reprod. 4: 208–214.

Quinn, J. A. 1987. Complex patterns of genetic differentiation and phenotypic plasticity versus an outmoded ecotype terminology. Pp. 95–111 *in* K. M. Urbanska (ed.), Differentiation Patterns in Higher Plants. Academic Press.

_____ and J. C. Colosi. 1977. Separating genotype from environment in germination ecology studies. Amer. Midl. Naturalist 97: 485–489.

Rathcke, B. and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. Ann. Rev. Ecol. Syst. 16: 179–214.

Reznick, D. N. 1985. Cost of reproduction: an evaluation of the empirical evidence. Oikos 44: 257–267.

Richardson, T. E. and A. G. Stephenson. 1992. Effects of parentage and size of the pollen load on progeny performance in *Campanula americana*. Evolution 46: 1731–1739.

Roach, D. A. and R. D. Wulff. 1987. Maternal effects in plants. Ann. Rev. Ecol. Syst. 18: 209–235.

Schaal, B. A. 1984. Life history variation, natural selection, and maternal effects in plant populations. Pp. 188–211 *in* R. Dirzo and J. Sarukhan (eds.), Perspectives on Plant Population Ecology. Sinauer, Sunderland, Massachussetts.

Schemske, D. W. 1984. Population structure and local selection in *Impatiens pallida* (Balsaminaceae), a selfing annual. Evolution 38: 817–832.

Schlichting, C. D. 1986. The evolution of phenotypic plasticity in plants. Ann. Rev. Ecol. Syst. 17: 667–693.

_____. 1989a. Phenotypic plasticity in *Phlox*. II. Plasticity of character correlations. Oecologia 78: 496–501.

_____. 1989b. Phenotypic integration and environmental change. BioScience 39: 460–464.

_____ and D. A. Levin. 1986. Effects of inbreeding on phenotypic plasticity in cultivated *Phlox*. Theor. Appl. Genet. 72: 114–119.

Schmitt, J. 1983. Individual flowering phenology, plant size, and reproductive success in *Linanthus androsaceus,* a California annual. Oecologia 59: 135–140.

_____. 1993. Reaction norms of morphological and life history traits to light availability in *Impatiens capensis*. Evolution 47: 1654–1668.

_____ and J. Antonovics. 1986. Experimental studies of the evolutionary significance of sexual reproduction. III. Maternal and paternal effects during seedling establishment. Evolution 40: 817–829.

_____, J. Eccleston, and D. W. Ehrhardt. 1987a. Density-dependent flowering phenology, outcrossing, and reproduction in *Impatiens capensis*. Oecologia 72: 341–347.

_____, _____, and _____ . 1987b. Dominance and suppression, size-dependent growth, and self-thinning in a natural *Impatiens capensis* population. J. Ecol. 75: 651–655.

_____ and D. W. Ehrhardt. 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. Evolution 44: 269–278.

_____ and S. E. Gamble. 1990. The effect of distance from the parental site on offspring performance in *Impatiens capensis*: a test of the local adaptation hypothesis. Evolution 44: 2022–2030.

_____, J. Niles, and R. D. Wulff. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. Amer. Naturalist 139: 451–466.

_____ and R. D. Wulff. 1993. Light spectral quality, phytochrome, and plant competition. Trends Ecol. Evol. 8: 47–51.

Shaw, R. G. 1986. Response to density in a wild population of the wild herb *Salvia lyrata*: variation among families. Evolution 40: 492–505.

Simpson, R. L., M. A. Leck, and V. T. Parker. 1985. The comparative ecology of *Impatiens capensis* Meerb. (Balsaminaceae) in central New Jersey. Bull. Torrey Bot. Club 112: 295–331.

Smith, B. H. 1984. The optimal design of a herbaceous body. Amer. Naturalist 123: 197–211.

Stearns, S. C. 1989. Trade-offs in life history evolution. Funct. Ecol. 3: 259–268.

_____, G. de Jong, and B. Newman. 1991. The effects of phenotypic plasticity on genetic correlations. Trends Ecol. Evol. 6: 122–126.

Stephenson, A. G. 1992. Factors that affect pollen performance. Pp. 119–136 *in* R. Wyatt (ed.), Ecology and Evolution of Plant Reproduction. Chapman and Hall, New York.

Stewart, S. C. and D. J. Schoen. 1987. Patterns of phenotypic viability and fecundity selection in natural populations of *Impatiens pallida*. Evolution 41: 1290–1301.

Stratton, D. A. 1989. Competition prolongs expression of maternal effects in seedlings of *Erigeron annuus* (Asteraceae). Amer. J. Bot. 76: 1646–1653.

Sultan, S. E. 1987. Evolutionary implications of phenotypic plasticity in plants. Evol. Biol. 21: 127–178.

_____. 1990. Evolutionary implications of phenotypic plasticity: genetic diversity for norms of reaction to resource gradients in *Polygonum persicaria*. Ph. D. thesis, Harvard University.

Thompson, J. 1991. Phenotypic plasticity as a component of evolutionary change. Trends Ecol. Evol. 6: 246–249.

van Tienderen, P. H. 1990. Morphological variation in *Plantago lanceolata*: Limits of plasticity. Evol. Trends Pl. 4: 35–43.

_____. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. Evolution 45: 1317–1331.

Venable, D. L. and A. Búrquez. 1989. Quantitative genetics of size, shape, life history, and fruit characteristics of the heteromorphic composite *Heterosperma pinnatum*. I. Variation within and between populations. Evolution 43: 113–124.

_____ and _____. 1991. Quantitative genetics of size, shape, life history, and fruit characteristics of the heteromorphic composite *Heterosperma pinnatum*. II. Correlation structure. Evolution 44: 1748–1763.

_____, _____, G. Corral, E. Morales, and F. Espinosa. 1987. The ecology of seed heteromorphism in *Heterosperma pinnatum* in central Mexico. Ecology 68: 65–76.

Via, S. and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39: 505–522.

Wade, M. J. and S. Kalisz. 1990. The causes of natural selection. Evolution 44: 1947–1955.

Waller, D. M. 1980. Environmental determinants of outcrossing in *Impatiens capensis* (Balsaminaceae). Evolution 34: 747–761.

Watson, M. A. 1984. Developmental constraints: effects on population growth and patterns of resource allocation in a clonal plant. Amer. Naturalist 123: 411–426.

Weis, A. E., H. G. Hollenbach, and W. G. Abrahamson. 1987. Genetic and maternal effects on seedling characters of *Solidago altissima* (Compositae). Amer. J. Bot. 74: 1476–1486.

Willis, J. H. 1991. The role of inbreeding depression in the evolution of two partially self-fertilizing populations of *Mimulus guttatus*. Ph. D. thesis, University of Chicago.

Wulff, R. D. 1986. Seed size variation in *Desmodium paniculatum*. I. Factors affecting seed size. J. Ecol. 74: 87–98.

_____ and F. A. Bazzaz. 1992. Effect of the parental nutrient regime on growth of the progeny in *Abutilon theophrasti* (Malvaceae). Amer. J. Bot. 79: 1102–1107.

Young, H. J. and M. L. Stanton. 1990. Influence of environmental quality on pollen competitive ability in wild radish. Science 248: 1631–1633.

GENE FLOW AMONG PLANT POPULATIONS: EVIDENCE FROM GENETIC MARKERS[1]

J. L. Hamrick[2], Mary Jo W. Godt[2], and Susan L. Sherman-Broyles[2,3]

ABSTRACT. Gene flow among populations and population subdivisions can have significant effects on the spatial distribution of genetic variation. Yet it has been difficult to obtain accurate estimates of gene flow from natural plant populations. Indirect measures of gene flow can be obtained from the distribution of genetic diversity among populations (i.e., F_{ST} values) or from the frequency of alleles restricted to single populations. A review of the plant allozyme literature indicates that indirect estimates of gene flow vary widely among species, but that species with similar traits tend to have similar rates of gene flow (e.g., outcrossing, wind-pollinated species have the highest rates while selfing species have the lowest). The identification of single gene markers or multilocus gametes that have immigrated into recipient populations allows direct estimates of gene flow rates. Direct estimates of gene flow based on multilocus analyses indicate that gene flow may be extensive at distances of over 1000 m in some plant species. Thus, the direct and indirect estimates of gene flow are generally consistent. Gene flow estimates obtained from individuals within populations vary widely indicating that, at this level, gene flow is highly idiosyncratic, perhaps due to such factors as flower density, plant size, flowering phenology and spatial position within the population.

Gene flow is defined as the movement of genes from one population or population subdivision to another. Population genetics theory has shown that if the number of migrants per generation (*Nm*, where *N* is the number of individuals in a population and *m* is the rate of gene flow) exceeds 4, gene flow will counteract genetic drift within populations (Wright, 1931; Slatkin, 1985a). In contrast, if *Nm* is less than 1 genetic drift can be the dominant factor shaping genetic structure. Thus, quantitative estimates of gene flow and descriptions of gene flow patterns are essential for understanding genetic differentiation. Without such estimates it is impossible to discuss the effects of selection and genetic drift on population structure or to understand local and geographic patterns of genetic variation.

Given the potential importance of gene flow in counteracting genetic drift and natural selection (Jain and Bradshaw, 1966) it is surprising how little quantitative data are available concerning gene flow in natural plant populations (Hamrick, 1987). As a result, we have only a rudimentary understanding of gene movement patterns within plant populations (i.e., the breeding structure of plant populations) and we know even less about gene movement among plant populations. Our understanding of the relative importance of selection, drift and gene flow in shaping the genetic structure of plant populations is, therefore, seriously compromised.

The general scarcity of gene flow measurements cannot be attributed to a lack of interest, or to misconceptions regarding the evolutionary significance of gene flow. Since the 1960s there have been numerous attempts to describe gene movement within plant populations (see Levin and Kerster, 1974, for a review of earlier work). Most studies monitored the movement of pollen or seed vectors or followed uniquely marked pollen or

[1]We thank John Nason for his assistance with the production of the figures.
[2]Departments of Botany and Genetics, University of Georgia, Athens, GA 30602, U.S.A.
[3]Present address: Department of Biological Sciences, SUNY College at Cortland, P.O. Box 2000, Cortland, NY 13045, U.S.A.

seeds. The limitations of such techniques have been discussed in detail elsewhere (Hamrick, 1987). Occasionally, the movement of genetic markers was followed. A major drawback of such studies was that most were concerned with gene movement within populations (e.g., Müller, 1977; Handel, 1982, 1983) and did not directly address gene movement among populations. Nevertheless, patterns of gene movement within populations were often extrapolated to estimate gene flow among populations. Such estimates suggested that gene flow was limited and, thus, was of little importance in determining the distribution of genetic diversity (Ehrlich and Raven, 1969; Levin and Kerster, 1974).

The increased availability of genetic markers, particularly allozymes, and the recent development of analytical procedures currently permit gene flow estimates in a wide array of plant species under a variety of natural and experimental conditions. In this paper two general approaches to estimate gene flow rates are discussed: indirect measures based on the distribution of genetic diversity among plant populations and direct measures estimated from the appearance of foreign alleles or gametes in a genetically defined population.

INDIRECT MEASURES OF GENE FLOW

Two indirect methods of estimating gene flow are currently used. The first method is based on the relationship between the degree of genetic differentiation among populations or population subdivisions as measured by Wright's F_{ST} statistic and the number of migrants per generation (Nm). Thus,

$$Nm = (1 - F_{ST})/4F_{ST}$$

where $F_{ST} = \sigma_p^2 / [\bar{p}_i(1 - \bar{p}_i)]$ and $\bar{p}_i$ is the mean frequency of allele i across all the populations (Wright, 1931, 1951). This relationship is based on Wright's infinite island model and assumes selection has not influenced population differentiation. With the exception of these assumptions, this is an attractive procedure since data from all studies of genetic diversity in plant populations can be used to estimate Nm. If only a few populations have been analyzed, Crow and Aoki (1984) have shown that the infinite island model can be approximated by dividing the Nm value obtained from Wright's equation by $[k/(k - 1)]^2$, where k is the number of populations studied. Nei's (1973) G_{ST} statistic, which partitions genetic diversity for multiallelic loci, can be substituted for Wright's F_{ST}.

The second method of calculating Nm from genetic diversity data is based on an observation by Slatkin (1980) that the conditional average frequencies of alleles (i.e., the mean frequencies of alleles in populations in which they occur) are independent of all evolutionary factors except gene flow. Slatkin (1985b) and Barton and Slatkin (1986) developed an empirical relationship that obtains quantitative estimates of Nm based on the mean frequency of alleles found in a single population (i.e., private alleles). This relationship is:

$$\log_{10}[\bar{p}(1)] = a \log_{10}(Nm) + b$$

where $\bar{p}(1)$ is the mean frequency of private alleles and a and b are constants that vary with sample size (Barton and Slatkin, 1986).

Slatkin and Barton (1989) compared the F_{ST} and private allele methods for utility and statistical robustness. Both methods provide reasonably accurate estimates of Nm under a wide variety of conditions. However, Slatkin and Barton (1989) noted that the F_{ST} based method is empirically preferable since it uses all the gene frequency data, and, thus, is less sensitive to errors in the interpretation of electrophoretic gels. Also, for most plant species the number of polymorphic loci available for estimates of F_{ST} is greater than the number of private alleles.

We reviewed the plant allozyme literature to obtain estimates of *Nm* based on the F_{ST} and private allele methods. In some cases the author(s) had estimated these values but in many cases we calculated one or both of these parameters from the allele frequency data. Each taxon reviewed was classified for eight life history or ecological traits (Table 1). Mean *Nm* values were calculated for each category of the eight traits. Data were analyzed using the GLM procedures of SAS (1988).

Table 1. A summary of the relationships between indirect measures of gene flow (*Nm*(*W*)) and eight life history and ecological traits. See Hamrick and Godt (1989) for a listing of the categories for each trait. Highly significant ($P < 0.001$) differences were found for each trait except mode of reproduction.

Trait	Low Gene Flow	High Gene Flow
Taxonomic Status	Dicot	Gymnosperm
Geographic Range	Endemic	Regional
Regional Distribution	Tropical	Boreal-Temperate
Life Form	Annual	Long-lived Woody
Mode of Reproduction	Not Significant	
Breeding System	Selfing	Outcrossed-Wind
Seed Dispersal	Gravity	Wind
Successional Status	Early	Late

Estimates of *Nm* based on the private alleles procedure were available for 296 taxa, while 644 taxa provided estimates of *Nm* from F_{ST} values. The *Nm* values from private alleles (*Nm*(*S*)) averaged 3.53 across all taxa while values derived from F_{ST} (*Nm*(*W*)) averaged 3.08. The correlation between *Nm*(*S*) and *Nm*(*W*) values was highly significant ($P < 0.001$) but low ($r = 0.32$). Statistically significant differences in gene flow rates existed among categories of seven of the eight life history and ecological traits (Table 1). Only mode of reproduction (sexual vs. asexual) had non-significant differences in *Nm* among its categories. Species with high rates of gene flow included gymnosperms, species with large geographic ranges, northern species, long-lived woody species, wind-pollinated, outcrossing species, species with wind-dispersed seeds and species of later successional stages. Species with the lowest rates of gene flow included dicots, endemic species, tropical species, annuals, selfing species, species with gravity-dispersed seeds and species of early successional stages. These patterns are consistent with results of earlier reviews of the allozyme literature (Loveless and Hamrick, 1984; Govindaraju, 1988; Hamrick and Godt, 1989).

We examined in greater detail several traits that are of special interest to plant evolutionary biologists because of their potential effects on gene flow. These traits were geographic range, breeding system and seed dispersal mechanism. To examine geographic range we grouped species into four categories: endemic, narrow, regional, and widespread.

Endemic species have ranges limited to relatively few populations in a localized area (e.g., northwestern California). Species classified as having narrow ranges generally have more populations distributed across a larger geographic region (i.e., one or two states). Regionally distributed species occur over much larger areas (e.g., western United States), while species classified as widespread occur on at least two continents.

Endemic species had the lowest mean *Nm* values, although *Nm*(*S*) values were not significantly different among categories (Table 2). Focussing on *Nm*(*W*) estimates, endemic and narrowly distributed species had significantly lower mean rates of gene flow than regionally distributed species. Widespread species, on the other hand, had a mean *Nm*(*W*) value that was intermediate between that of regionally distributed species and species with smaller ranges. Species with limited distributions may tend to occur in smaller, more isolated populations while species with widespread distributions may be more likely to occur in larger, more continuous populations. Thus, the movement of genes from one geographic area to another is more likely in species with continuous distributions. However, the mean *Nm* value for all four geographic range categories was well above 1.0, indicating that gene flow can play a significant role in patterning genetic diversity regardless of the species' geographic range.

Table 2. Mean indirect estimates of gene flow (*Nm*(*S*) and *Nm*(*W*)) for species with different geographic ranges. Values sharing the same letter are not significantly different at the $P \leq 0.05$ level.

Geographic Range	Number of Species	*Nm*(*S*)	Number of Species	*Nm*(*W*)
Endemic	28	1.58[a]	70	1.96[a]
Narrow	73	4.62[a]	151	2.14[a]
Regional	138	3.35[a]	295	3.86[b]
Widespread	57	3.41[a]	128	3.03[ab]

Major differences in *Nm* appeared among the five breeding system categories (Table 3). Seventy-four percent of the selfing species had *Nm*(*W*) values < 1.0 (Fig. 1). Estimated values of *Nm* were intermediate for the mixed-mating species and for the outcrossed, animal-pollinated species but were well above 1.0. Although the mean *Nm* values of these two categories were not significantly different, distributions of their *Nm*(*W*) values were quite different (Fig. 1). Only 10% of the mixed-mating, wind-pollinated species had *Nm*(*W*) values < 1.0 while 41% of the animal-pollinated, mixed-mating species had *Nm*(*W*) values below this level. Among the predominantly outcrossing species, wind-pollinated species had significantly higher mean *Nm*(*W*) values than animal-pollinated species. Seven percent of the wind-pollinated outcrossing species had *Nm*(*W*) values < 1.0 while 24% of the animal-pollinated, outcrossing species had *Nm*(*W*) values below this threshold.

Table 3. Mean indirect estimates of gene flow ($Nm(S)$ and $Nm(W)$) for species with different breeding systems. Values sharing the same letter are not significantly different at the $P \leq 0.05$ level.

Breeding System	Number of Species	$Nm(S)$	Number of Species	$Nm(W)$
Selfing	47	0.89^{a}	108	0.57^{a}
Mixed-Animal	44	2.26^{a}	83	1.61^{ab}
Mixed-Wind	3	3.61^{ab}	14	1.52^{ab}
Outcrossed-Animal	93	3.21^{ab}	220	2.33^{b}
Outcrossed-Wind	109	5.46^{b}	219	5.73^{c}

The results of the breeding system analysis were not unexpected since reviews of the plant allozyme literature have consistently shown the breeding system to be a major factor influencing the distribution of genetic diversity. The present data, which are the most comprehensive to date, suggest that gene flow among populations of most predominantly selfing species is not high enough to prevent genetic drift from having a major impact on the distribution of genetic variation. For species with mixed-mating systems and animal-pollinated outcrossing species, genetic drift will play some role in determining genetic structure but gene flow appears to be high enough to prevent the loss of genetic diversity due to drift. Outcrossing, wind-pollinated species have a mean $Nm(W)$ value above 4.0 and, as a result, genetic drift should not play a major role in the genetic structure of such species.

Significant differences in Nm were also seen among the six seed dispersal categories (Table 4). Species with explosively dispersed seeds had the lowest mean Nm values while wind-dispersed species had the highest Nm values. Species with gravity-dispersed seeds and either animal-attached or secondarily animal-dispersed seeds had mean Nm values that were not significantly higher than those of explosively dispersed species. Species with animal-ingested seed dispersal had a mean $Nm(W)$ value that was significantly less than the wind-dispersed species but only significantly higher than the gravity-dispersed species.

Although the results of the seed dispersal analysis are generally consistent with *a priori* expectations it is somewhat surprising that species with animal-ingested seed dispersal mechanisms have significantly lower $Nm(W)$ values than wind-dispersed species. There are, perhaps, several reasons for this result. First, while the potential for very long distance movement may be higher for seeds ingested by animals, such long-distance dispersal events may make up a relatively minor percentage of seed movements. In addition, only a small percentage of the seeds moved long distances may be deposited in appropriate habitats. As a result, the modal movement for seeds of such species may be quite short. Wind-dispersed species may have less potential for very long distance seed movement but their modal movement distance may be greater than that for animal-ingested species. Also the more "wave-like" movement of wind-dispersed seeds might ensure that a greater number are deposited in suitable sites for germination and establishment.

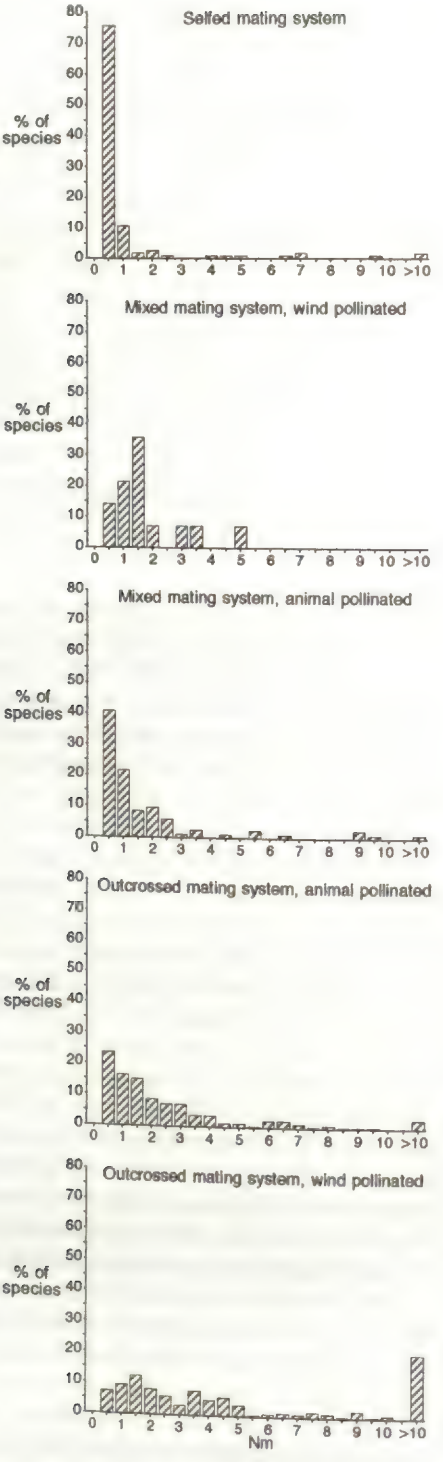

Selfed mating system
Mixed mating system, wind pollinated
Mixed mating system, animal pollinated
Outcrossed mating system, animal pollinated
Outcrossed mating system, wind pollinated
% of species
0 1 2 3 4 5 6 7 8 9 10 >10
Nm

Figure 1. Distribution of $Nm(W)$ values for species with different breeding systems. Sample sizes for each category are given in Table 3.

Table 4. Mean indirect estimates of gene flow ($Nm(S)$ and $Nm(W)$) for species with different seed dispersal mechanisms. Values sharing the same letter are not significantly different at the $P \leq 0.05$ level.

Seed Dispersal	Number of Species	$Nm(S)$	Number of Species	$Nm(W)$
Gravity	121	1.98[a]	266	1.69[a]
Explosive	10	1.58[ab]	24	1.27[ab]
Gravity-Animal	13	4.67[ab]	16	2.12[ab]
Animal-Attached	33	2.35[ab]	71	2.52[ab]
Animal-Ingested	30	5.94[b]	54	3.24[b]
Wind	89	5.33[b]	213	5.24[c]

An alternate explanation is that a higher proportion of the long-distance moves of animal-ingested seeds may result in the colonization of new habitats rather than in gene flow between established populations. The theoretical research of Slatkin (1977), Wade and McCauley (1988), and McCauley (1991) has demonstrated that dispersal/colonization events produce more differentiation among populations (i.e., higher F_{ST} values and lower estimates of Nm) than gene flow among established populations. The actual proportion of seeds involved in colonization versus gene flow events needs empirical investigation before definitive conclusions can be reached.

Plant species, therefore, vary greatly in their potential for gene movement among populations. As expected, species with limited potential for seed and pollen movement have quite low Nm values. On the other hand, species with wind-pollination and wind-dispersed or animal-ingested seeds have rates of gene flow high enough to counteract genetic drift. It is noteworthy that more than 70% of the plant species ($N = 644$) included in this review have Nm values greater than 1.0 (Fig. 1). Thus, genetic drift should not play a major role in the genetic structuring of populations of most outcrossing species.

Finally, it is notable that many of the extreme values of $Nm(W)$ within any particular category (Fig. 1) were due to F_{ST} estimates that were based on very few polymorphic loci. This suggests that the reliability of $Nm(W)$ estimates improves with the number of variable loci used. Thus, care should be used in the interpretation of Nm values for individual species. Nevertheless, we conclude that particular suites of characteristics are significantly associated with differing levels of gene flow.

DIRECT ESTIMATES OF GENE FLOW

Direct estimates of gene flow are based on differences in allele frequencies between adult and progeny generations or on the presence of an allele or a multilocus gamete in progeny that is absent in the adult population. The latter situation is preferable since the

genetic composition of the progeny provides an unambiguous estimate of the minimum rate of gene flow ('apparent' gene flow).

Single gene markers: Estimates of gene flow rates can be made from differences in allele frequencies at single loci by comparing adult allele frequencies with those of their progeny. Thus,

$$m = (q_1 - q_o) / (Q - q_o)$$

where m is the rate of gene flow, q_1 is the allele frequency in the progeny generation, q_0 is the allele frequency in the adult generation and Q is the mean allele frequency in surrounding populations. This estimation procedure assumes that differences in allele frequencies between adult and progeny generations are due only to gene flow. This assumption is difficult to test since many factors can cause changes in allele frequencies. Examples of such factors are differential reproduction among adults or selection between fertilization and time of analysis. Because of the necessity of this untestable assumption, single gene estimates of gene flow based on allele frequency differences have limited use in most experimental or natural situations. However, this assumption is unnecessary if an allele absent from the adults is observed in the population's progeny. The presence of such an allele can be unambiguously attributed to gene flow. However, alleles that occur in relatively high frequencies generally occur in all populations.

In most experimental studies of gene flow researchers have placed a group of genetically marked individuals in the center of an array of genotypes homozygous for an alternate allele. The appearance of the marker allele in the progeny of surrounding individuals describes gene movement patterns within the experimental populations. Schaal's (1980) study comparing pollinator movement distances and actual patterns of gene movement in an experimental *Lupinus texensis* population is an excellent example of such a study. Schaal introduced seven plants marked with a unique PGI allele into an array of 91 plants having alternate alleles. She monitored pollinator moves as well as the presence of the marker allele in progeny of surrounding individuals. Her results showed that although the distributions of both pollinator movements and the marker allele were leptokurtic, the mean distance of gene movement was approximately twice the mean pollinator movement, indicating that pollen carryover was substantial.

In natural populations single gene markers have been primarily used to describe the movement of genes within populations rather than gene flow among populations. This is due to the rarity of monomorphic populations or population subdivisions. Single gene markers have proved more useful in the study of gene flow between artificially established populations monomorphic for alternate alleles (e.g., Bateman, 1947; Smyth and Hamrick, 1987). Domesticated species have commonly been used because of well-known polymorphisms, the easy availability of stock, rapid generation time and ease of cultivation. An excellent example is the work of Bateman (1947), who used single gene morphological markers to study gene movement among experimental populations of two crop species, *Raphanus sativus* L. and *Brassica rapa* L. Pooling data from the two species, Bateman (1947) obtained mean gene flow rates of 6% between populations separated by 42 m and 1% among populations separated by 170 m.

The use of single gene marker loci in natural populations has usually been limited to descriptions of movements of a rare allele from one or a few individuals (usually heterozygotes). Rare alleles are of limited use in estimating gene flow among populations since the low frequency of the allele in the source population makes it virtually impossible to observe in the recipient population without analyzing an extremely large number of progeny. Under fortuitous circumstances one can occasionally discover a natural population or

population subdivision that is monomorphic at a locus with a moderate level of genetic diversity in surrounding populations. In such cases, progeny of the monomorphic population can be examined for heterozygotes. Just such a situation existed in the 1984 flowering cohort of *Tachigali versicolor* Standl. & L. W. Wiens, a large, leguminous, monocarpic tree that is relatively common in the moist tropical forest of Barro Colorado Island (BCI), Panama (Foster, 1977). In 1984, 5 of the 60 flowering individuals were isolated from other flowering individuals by at least 500 m. These five individuals were monomorphic for the most common allele at an IDH locus. The alternate allele had a frequency of 0.156 on BCI as a whole. When approximately 48 progeny were analyzed from each of the 5 isolated trees the less common allele occurred at a frequency of 4%. Using the equation given above (where $q_o = 0$; $q_1 = 0.04$; $Q = 0.156$), the gene flow rate into the isolated individuals was estimated as 25.6% (Hamrick and Loveless, 1989).

In conclusion, single gene markers can provide valuable information to better understand gene flow. When used in properly designed experimental populations, single marker alleles can elucidate both the patterns and the magnitude of gene flow. More experimental studies of this sort should be attempted since it is only through the use of controlled experimental populations that we will fully understand the effects of ecological factors (e.g., flower density, dispersion and intervening vegetation) on estimated rates of gene flow. In natural populations the use of single gene markers will be limited to situations where monomorphic populations or subpopulations can be identified.

Multilocus estimates of gene flow: Multilocus procedures for the estimation of gene flow are based on the same concepts as single-locus estimation procedures. But instead of examining progeny for the presence of an immigrant allele, progeny are examined for multilocus gametes that could not have been produced by the reproductive adults. This is a particularly productive approach for gymnosperms since the maternal gamete can be determined directly by analyzing the haploid megagametophytic tissue that surrounds the embryo. Given the female gamete and the embryonic genotype, the genotype of the male gamete can be determined through subtraction. This gamete can then be compared to the gametic arrays potentially produced by each adult. If it is genetically impossible for any adult to have produced the gamete in question, an apparent gene flow event has occurred. Estimates of total gene flow (apparent plus genetically cryptic gene flow) are given by

$$m = q_s/q_m$$

where m is the rate of gene flow, q_s is the frequency of a multilocus gamete in the recipient progeny population and q_m is the frequency of the multilocus gamete in surrounding populations (Friedman and Adams, 1985).

In angiosperms the estimation of gene flow is more complex. First, as in gymnosperms, foreign gametes and local gametes can be indistinguishable. Second, in angiosperms the female gamete can only be determined at homozygous loci and at loci for which the progeny carries a non-maternal allele. When heterozygous progeny have the same genotype as their heterozygous mother either allele can be assigned to the father. Thus, in general, the number of heterozygous loci within maternal individuals compromises our ability to specify the paternal gamete and diminishes our ability to detect immigrant gametes.

Several parameters must be determined to estimate total gene flow in angiosperms. First, estimates of maternal and paternal allele frequencies must be determined for the study population. Next, allele frequencies for the surrounding populations must be accurately

estimated. Finally, paternity analysis techniques must be used to identify the number of multilocus gametes that could not have been produced by adults in the target populations (i.e.,apparent gene flow events). The ability to identify several apparent gene flow events is influenced by the genetic diversity of the species (i.e., the number of polymorphic loci, the number of alleles per locus and allele frequencies), the number of potential pollen donors in the recipient population, the actual level of gene flow, and the number of progeny sampled. Populations with high genetic diversity and a limited number of adults are ideal since there is a high probability of identifying several apparent gene flow events.

Devlin and Ellstrand (1990) developed an analytical procedure that estimates total gene flow from the angiosperm data described above. First, a parameter ϕ, the probability that a gamete produced by the surrounding population could *not* be produced by an individual in the recipient population, is calculated from the product of allele frequencies in the surrounding populations. It can be shown that the ratio $\phi/(1 - \phi) = x/y$ where x is the apparent rate of gene flow and y is cryptic gene flow. The sum $x + y$ is a first estimate of total gene flow by pollen but is an underestimate because of the problems discussed in the preceding paragraphs. To circumvent this problem, Devlin and Ellstrand (1990) developed a Monte Carlo method that estimates the total number of gene flow events.

The Devlin and Ellstrand (1990) procedure has several assumptions. First, plants surrounding the recipient population are viewed as a single large population. Second, successful immigrant gametes received by the recipient population are assumed to be a random sample of gametes from individuals in the outlying populations. Finally, the loci are assumed to be in linkage equilibrium.

Multilocus estimates of gene flow are increasing in the plant evolutionary literature. An example is the study by Hamrick and Murawski (1990, and unpublished data) of a tropical leguminous tree, *Platypodium elegans* J. Vogel. The study area was a 50-ha mapped plot on Barro Colorado Island (BCI), Panama, surrounded by a 100-m buffer zone (84 ha total) within which all mature *P. elegans* were identified. The recipient population consisted of 37 mature trees located within the internal 50-ha plot. Seeds were collected from each flowering tree over a three-year period (1987–89). Each progeny and every adult in the 84-ha area was analyzed for 15 polymorphic allozyme loci. Surveys of six collection sites located throughout BCI (Hamrick and Loveless, 1989) were used to provide estimates of allele frequencies for the surrounding populations. A fractional paternity analysis (Devlin et al., 1988) was used to identify progeny for which no father could be identified within the study area. The estimation procedure of Devlin and Ellstrand (1990) was then used to determine overall rates of gene flow during each year.

Gene movement within the recipient population was approximately leptokurtic during each of the three years (Fig. 2). Roughly 20% of the gene movements were beyond 750 m and mean gene movement ranged from 369 m in 1989 to 420 m in 1988 (Table 5). The most remarkable finding was that overall rates of gene flow from outside the 84-ha area were greater than 60% in two out of the three years. It should be remembered, however, that even within the 84-ha area (1200 m × 700 m) the greatest distance from the boundary of the study area to the most centrally located maternal individual was 350 m, well within the range of gene movement observed within the study plot. These results, coupled with those described earlier for *Tachigali versicolor,* indicate that insect-pollinated tropical trees may experience rather high levels of gene flow—often over distances greater than 500 m. These direct estimates of gene flow are consistent with estimates of Nm based on the distribution of genetic diversity among collection sites separated by at least one kilometer on BCI ($Nm(W)$ = 3.2 for *P. elegans* and 3.3 for *T. versicolor*, Hamrick and Loveless, 1989).

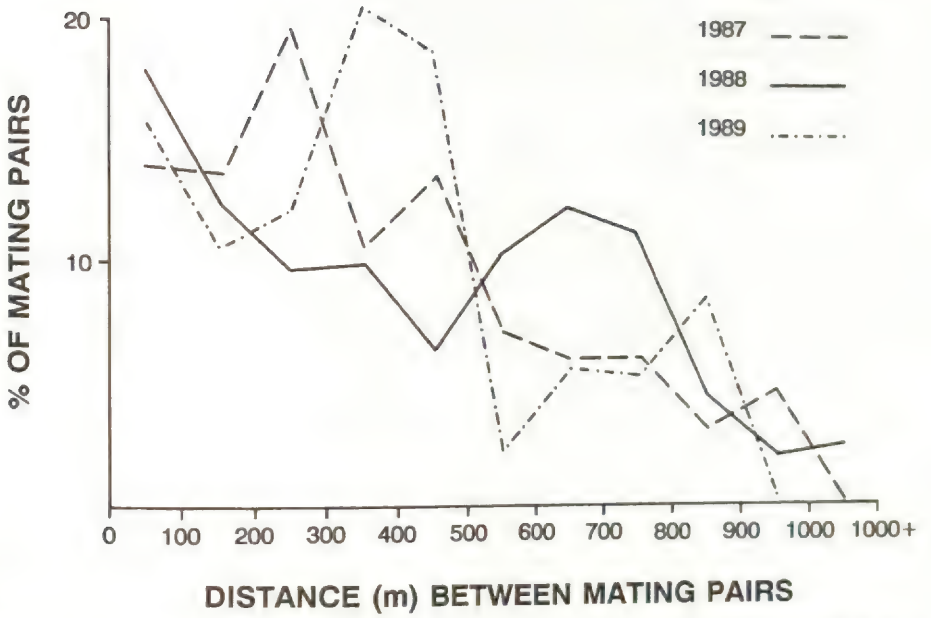


Figure 2. Distribution of gene movement among *Platypodium elegans* adults located in a 700 m × 1200 m plot on Barro Colorado Island, Panama (after Hamrick and Murawski, 1990).

Table 5. Percentage of *Platypodium elegans* seeds sired by foreign pollen entering an 84-ha study area during three years. Apparent gene flow was estimated by paternity exclusion analysis. Total gene flow was estimated by the Monte Carlo method of Devlin and Ellstrand (1990) and cryptic gene flow was obtained by subtraction (total - apparent = cryptic gene flow).

Year	N	Mean Distance (m)	Apparent Gene Flow	Cryptic Gene Flow	Total Gene Flow (95% Confidence Level)
1987	269	374.5	19.0%	20.1%	39.1% (31.0–47.2%)
1988	116	419.6	39.7%	26.7%	66.4% (54.6–72.4%)
1989	163	368.8	45.1%	32.2%	77.3% (65.6–89.0%)

Godt and Hamrick (1993) examined gene flow in *Lathryus latifolius* L. (everlasting pea) located in Athens, Georgia. *Lathryus latifolius* is a perennial leguminous vine that is primarily pollinated by bumblebees. Ten polymorphic allozyme loci were used to determine the number of apparent gene flow events into two sites separated from surrounding populations by approximately 30 m. Allele frequencies of flowering individuals in the surrounding populations were determined, permitting estimation of total gene flow into these two sites.

The estimated proportion of migrant alleles ranged from 16% in Site B in 1988 to 46% in Site A during 1989 (Table 6). Estimated gene flow was higher in 1989 and may have been influenced by the lower density of flowering individuals during that year. Rates of gene flow also varied greatly among individual adults. This variation was not correlated with the position of adults in the study sites, suggesting that at the individual level gene flow can be quite idiosyncratic. Earlier observations using single gene markers have also shown this to be the case (Handel, 1983; Smyth and Hamrick, 1987).

Table 6. Percentage of seeds sired by foreign pollen entering two *Lathyrus latifolius* sites during two years. Apparent gene flow was estimated by paternity exclusion analysis, total gene flow was estimated by the Monte Carlo method of Devlin and Ellstrand (1990) and cryptic gene flow was obtained by subtraction (total - apparent = cryptic gene flow). N is the number of progeny analyzed. From Godt and Hamrick (1993).

Year/Site	N	Apparent Gene Flow	Cryptic Gene Flow	Total Gene Flow (95% Confidence Interval)
1988				
Site A	414	7.7%	25.5%	33.2% (29.7–36.7)
Site B	980	4.7%	11.1%	15.8% (14.5–17.1)
1989				
Site A	454	15.4%	30.4%	45.8% (42.8–48.9)
Site B	819	9.6%	20.9%	30.5% (28.8–32.3)
Means				
Site A		11.6%	27.9%	39.5%
Site B		7.2%	15.9%	23.1%
Overall		9.3%	22.0%	31.3%

The final example is a study of gene flow into six populations of *Asclepias exaltata* L. (poke milkweed) located in Shenandoah National Park, Virginia (Broyles, 1992). Milkweeds are particularly good study organisms for gene flow estimation since seeds from individual fruits are usually sired by pollen from a single paternal individual (Broyles and Wyatt, 1990). The correlated mating system and the presence of numerous seeds per fruit make it possible to determine the paternal genotype. If an individual with this genotype does not exist in the population, all the seeds in that fruit can be attributed to gene flow. Furthermore, since the likelihood of having identical multilocus genotypes in the recipient and in the surrounding populations is remote, the ratio of apparent to total gene flow is much higher than that obtained for species with more conventional breeding systems. This should provide estimates of gene flow with much smaller standard errors.

The proportion of immigrant pollen in these *A. exaltata* populations ranged from 28.6% to 50.0% (Table 7) with a mean of 37.0% (Broyles, 1992). There was a rough negative association between the isolation distance (50 to 1000 m) and the level of gene flow into a population. Certainly the level of gene flow among populations of this insect-pollinated species indicates that migrant genes make up a significant proportion (18.5%) of the genes in the progeny.

Table 7. Pollen-mediated gene flow in *Asclepias exaltata* (Broyles, 1992).

Population	No. of Fruits Analyzed	No. of Seeds Analyzed	Percent Immigrant Pollen	Minimum Isolation Distance (m)
South Skyland	35	420	28.6%	800
Fisher's Gap	24	288	29.2%	1000
Skyland Lodge	44	528	31.8%	200
Mile Marker 54	58	696	37.9%	50
Old Rag Overlook	56	672	44.6%	400
Lewis Mountain	22	264	50.0%	50
Total	239	2,868	---	---
Mean	---	---	37.0%	500

Since the sizes of these milkweed populations were known, direct estimates of Nm could be calculated and compared to estimates made from the distribution of allele frequencies among these six populations ($Nm(W)$). The direct measure of Nm was 8.8 while the $Nm(W)$ value was 4.2 (Broyles, 1992). Both values indicate that gene flow is high enough to prevent genetic drift from playing a major role in the genetic structure of these populations.

DISCUSSION AND CONCLUSIONS

Both indirect and direct estimation procedures indicate that gene flow can be substantial in predominantly outcrossing plant species. The few examples that permit comparison of direct and indirect estimates of gene flow are encouraging in that the two estimates are relatively similar. However, there are several reasons why indirect and direct estimates might differ to some degree. First, most of the direct estimates are based only on pollen movement whereas indirect estimates should incorporate gene flow via pollen and seeds. Second, direct estimates of pollen movement are obtained from seeds taken directly from maternal individuals while indirect estimates are usually obtained from allele frequencies of established individuals. Thus, any selection against immigrant genes that might occur between the seed and more mature stages (e.g., Antonovics, 1968; Waser and Price,

1983) would tend to produce lower estimates of gene flow. Finally, the indirect estimates represent a composite of the effects of several generations of gene flow, while direct estimates represent measurements of gene flow during a very restricted period of time (e.g., during one year).

Estimation procedures that utilize multilocus analyses suggest that gene flow is much more extensive (Table 8) than previously thought (Ehrlich and Raven, 1969; Levin and Kerster, 1974). Why then are these results in such sharp contrast to earlier studies that indicated limited gene flow? There are several possible explanations. First, as Schaal (1980) and others (e.g., Thomson and Plowright, 1980) have shown, estimates of pollen movement that have not accounted for pollen carryover effects may seriously underestimate actual gene movement. Thus, pollen obtained from a plant may be deposited on several subsequently visited individuals. Furthermore, the amount of pollen deposited can vary greatly, with plants visited later in a sequence receiving more pollen from donor plants than plants visited earlier in the sequence (Thomson and Plowright, 1980; Lertzman and Gass, 1983).

Table 8. Multilocus estimates of gene flow and approximate distances of gene movement for several plant species.

Species (# Populations)	% Immigrant Pollen	Isolation Distance (m)	Authors
Asclepias exaltata (6)	29–50	50–1000	Broyles et al., 1993
Cucurbita foetidissima (16)	0–64	100–700	Kohn and Casper, 1992
Cucurbita texana (1)	5	> 1300	Kirkpatrick and Wilson, 1988
Gleditsia triacanthos (2)	40–70	> 200	Schnabel, 1988
Hordeum vulgare (1)	< 1	> 60	Wagner and Allard, 1991
Ipomopsis aggregata (3)	2–10	> 30	Campbell, 1991
Lathyrus latifolius (2)	16–46	≈ 30	Godt and Hamrick, 1993
Pinus taeda (2)	36–60	> 100	Friedman and Adams, 1985
Platypodium elegans (1)	36–77	> 100	Hamrick and Murawski, 1990
Raphanus sativus (3)	8–18	100–1000	Ellstrand and Marshall, 1985
Raphanus sativus (2)	6–7	> 150	Devlin and Ellstrand, 1990
Tachigali versicolor (1)	25	> 500	Loveless and Hamrick, unpubl. data

Second, early studies of pollen and seed movement were often limited to descriptions of movement patterns within rather than among populations. Usually little or no information was obtained about gene movement beyond the study area. Furthermore, the use of one or a few genetically marked individuals as the sole source of marker genes was subject to error due to highly idiosyncratic patterns of pollinator visitation and pollen deposition. Finally, the use of low frequency alleles as genetic markers necessitated the analysis of ever larger sample sizes as the distance away from the marked individual(s) increased. As one moved beyond the source population, sample sizes needed to locate the marker allele in a recipient population became prohibitive.

The beauty of the multilocus estimation procedures is that information on gene flow can be supplied by every fruiting individual in the population. This greatly increases the probability of observing a gene flow event and lowers confidence limits around gene flow estimates. In essence, multilocus estimation procedures have reversed the orientation of gene movement studies. Instead of tracking the movement of genes away from a point source, multilocus procedures utilize each flowering individual as a pollen trap for immigrant genes, permitting more robust estimates of gene flow.

This paper has reviewed gene movement via pollen and has largely neglected seed movement. This is for a good reason—most studies of gene flow in plants have focussed on pollen movement. Yet reviews of the allozyme literature (e.g., Hamrick and Godt, 1989) indicate that seed dispersal also has an important effect on genetic structure (Table 4). Seed movement has been largely neglected simply because successful seed dispersal is more difficult to monitor and to quantify than pollen movement. The use of multilocus techniques to study gene flow by seeds has the potential to rectify this situation somewhat, but even with these techniques the documentation of seed movement will be difficult.

There are two potential uses of multilocus procedures in the estimation of seed movement. The most straightforward approach would be to develop a set of genetic markers for maternally inherited genomes (chloroplast and mitochondria for most angiosperms, mitochondria for gymnosperms). With a sufficient number of polymorphic loci, seeds or seedlings could be sampled to determine if the maternally inherited genome was donated by an adult in the population. If not, the seed or seedling must be attributed to immigration. To date, however, cpDNA and mtDNA have not provided the marker loci needed for such analyses.

The second approach relies on nuclear genes to identify (by a modified paternity analysis) seeds or seedlings without possible parents within the study site. However, for a given level of genetic diversity the probability of excluding parent pairs is lower than the probability of excluding a paternal individual for progeny whose mother is known. Thus, to obtain reliable estimates of seed immigration either the number of progeny sampled must be larger than the number needed to measure pollen flow or the number of loci used in the parent analysis must be increased above that used in pollen movement studies. The use of RFLP or RAPD loci may make this a possibility in the future.

Indirect estimates of gene flow rates are associated with particular characteristics of plant species (i.e., geographic range, the breeding system and seed dispersal mechanisms). Selfing species experience little gene flow and the distribution of their genes may result from genetic drift. Species with outcrossing breeding systems and the potential for long-distance seed dispersal or with more continuous populations tend to have high rates of gene flow, and genetic drift should play a minor role in structuring their populations. Direct estimates of gene flow based on multilocus analyses are consistent with this conclusion (Table 8). The genetic ramifications of these differences in gene flow rates are great. Species with lower potentials

for gene flow tend to have fewer polymorphic loci and fewer alleles per polymorphic locus (Hamrick and Godt, 1989). Thus, the long-term evolutionary potential of these species may also be less.

These conclusions have applied implications. The high rates of gene flow in outcrossing species indicate that there are few barriers to the movement of genes from experimental populations containing genetically altered individuals into natural conspecific populations (Ellstrand and Hoffman, 1990). There are also implications for conservation biology, particularly the concern for the increased fragmentation of natural populations (Templeton et al., 1990). If gene flow is high, fragmented populations may continue to exchange genes. Such gene exchange would retard differentiation and the loss of genetic diversity within each fragment. This, in turn, would minimize the reduction of genetic diversity in species resulting from the subsequent destruction of fragments.

In conclusion, we believe that multilocus genetic analyses have only begun to describe the complexity of gene flow in natural plant populations. Direct estimates of gene flow for species with different suites of characteristics are needed to confirm the indirect estimates of gene flow and to provide information on variation in gene flow rates among plant species. We therefore eagerly anticipate the use of genetic markers to monitor temporal variation in gene flow rates and to elucidate the influence of ecological factors on gene flow by pollen *and* seeds.

LITERATURE CITED

Antonovics, J. 1968. Evolution in closely adjacent plant populations. VI. Manifold effects of gene flow. Heredity 23: 507–524.

Barton, N. H. and M. Slatkin. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity 56: 409–415.

Bateman, A. J. 1947. Contamination of seed crops. I. Insect pollination. J. Genet. 48: 257–275.

Broyles, S. B. 1992. Reproductive biology of poke milkweed, *Asclepias exaltata* L.: Population structure, mating patterns, pollen dispersal and the evolution of inflorescence size. Ph.D. Dissertation, University of Georgia. 206 pp.

_____ and R. Wyatt. 1990. Paternity analysis in a natural population of *Asclepias exaltata*: Multiple paternity, functional gender, and the "pollen-donation hypothesis." Evolution 44: 1454–1468.

Campbell, D. R. 1991. Comparing pollen dispersal and gene flow in a natural population. Evolution 45: 1965–1968.

Crow, J. F. and K. Aoki. 1984. Group selection for a polygenic behavioral trait: Estimating the degree of population subdivision. Proc. Natl. Acad. Sci. U.S.A. 81: 6073–6077.

Devlin, B. and N. C. Ellstrand. 1990. The development and application of a refined method for estimating gene flow from angiosperm paternity analysis. Evolution 44: 248–259.

_____, K. Roeder, and N. C. Ellstrand. 1988. Fractional paternity assignment: Theoretical development and comparison to other methods. Theor. Appl. Genet. 76: 369–380.

Ehrlich, P. R. and P. H. Raven. 1969. Differentiation of populations. Science 165: 1228–1232.

Ellstrand, N. C. and C. A. Hoffman. 1990. Hybridization as an avenue of escape for engineered genes. Bioscience 40: 438–442.

_____ and D. L. Marshall. 1985. Inter-population gene flow by pollen in wild radish, *Raphanus sativus*. Amer. Naturalist. 126: 606–616.

Foster, R. 1977. *Tachigalia versicolor* is a suicidal neotropical tree. Nature 268: 624–626.

Friedman, S. T. and W. T. Adams. 1985. Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.). Theor. Appl. Genet. 69: 609–615.

Godt, M. J. W. and J. L. Hamrick. 1993. Patterns and levels of pollen-mediated gene flow in *Lathyrus latifolius*. Evolution 47: 98–110.

Govindaraju, D. R. 1988. Relationship between dispersal ability and levels of gene flow in plants. Oikos 52: 31–35.

Hamrick, J. L. 1987. Gene flow and distribution of genetic variation in plant populations. Pp. 53–67 *in* K. Urbanska (ed.), Differentiation Patterns in Higher Plants. Academic Press, NY.

_____and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pp. 43–63 *in* A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir (eds.), Plant Population Genetics, Breeding, and Genetic Resources. Sinauer, Sunderland, MA.

_____and M. D. Loveless. 1989. The genetic structure of tropical tree populations: Associations with reproductive biology. Pp. 129–146 *in* J. H. Bock and Y. B. Linhart (eds.), The Evolutionary Ecology of Plants. Westview Press, Boulder, CO.

_____and D. A. Murawski. 1990. The breeding structure of tropical tree populations. Pl. Sp. Biol. 5: 157–165.

Handel, S. N. 1982. Dynamics of gene flow in an experimental garden of *Cucumis melo* (Cucurbitaceae). Amer. J. Bot. 69: 1538–1546.

_____. 1983. Pollination ecology, plant population structure, and gene flow. Pp. 163–211 *in* L. Real (ed.), Pollination Biology. Academic Press, NY.

Jain, S. K. and A. D. Bradshaw. 1966. Evolutionary divergence among adjacent plant populations. I. The evidence and its theoretical analysis. Heredity 21: 407–441.

Kirkpatrick, K. J. and H. D. Wilson. 1988. Interspecific gene flow in *Curcurbita: C. texana* vs. *C. pepo*. Amer. J. Bot. 75: 519–527.

Kohn, J. R. and B. B. Casper. 1992. Pollen-mediated gene flow in *Cucurbita foetidissima* (Cucurbitaceae). Amer. J. Bot. 79: 57–62.

Lertzman, K. P. and C. L. Gass. 1983. Alternative models of pollen transfer. Pp. 474–489 *in* C. E. Jones and R. J. Little (eds.), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold Company, NY.

Levin, D. A. and H. W. Kerster. 1974. Gene flow in seed plants. Evol. Biol. 7: 139–220.

Loveless, M. D. and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15: 65–95.

McCauley, D. E. 1991. Genetic consequences of local population extinction and recolonization. Trends Ecol. Evol. 6: 5–8.

Müller, G. 1977. Cross-fertilization in a conifer stand inferred from enzyme gene-markers in seeds. Silvae Genet. 26: 223–226.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. 70: 3321–3323.

SAS Institute, Inc. 1988. SAS/STAT™ User's Guide, Release 6.03 Edition. Sas Institute, Inc., Cary, NC. 1028 pp.

Schaal, B. 1980. Measurement of gene flow in *Lupinus texensis*. Nature 284: 450–451.

Schnabel, A. F. 1988. Population genetic structure and gene flow in *Gleditsia triacanthos* L. Ph.D. Dissertation, University of Kansas, Lawrence. 151 pp.

Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. Theor. Pop. Biol. 12: 253–262.

_____. 1980. The distribution of mutant alleles in a subdivided population. Genetics 95: 503–524.

_____. 1985a. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16: 393–430.

_____. 1985b. Rare alleles as indicators of gene flow. Evolution 39: 53–65.

_____ and N. H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43: 1349–1368.

Smyth, C. A. and J. L. Hamrick. 1987. Realized gene flow via pollen in artificial populations of musk thistle, *Carduus nutans* L. Evolution 41: 613–619.

Templeton, A. R., K. Shaw, E. Routman, and S. K. Davis. 1990. The genetic consequences of habitat fragmentation. Ann. Missouri Bot. Gard. 77: 13–27.

Thomson, J. D. and R. C. Plowright. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. Oecologia 46:68–74.

Wade, M. J. and D. E. McCauley. 1988. Extinction and recolonization: Their effects on the genetic differentiation of local populations. Evolution 42: 995–1005.

Wagner, D. B. and R. W. Allard. 1991. Pollen migration in predominantly self-fertilizing plants: Barley. J. Hered. 82: 302–304.

Waser, N. M. and M. V. Price. 1983. Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. Pp. 341–359 *in* C. E. Jones and R. J. Little (eds.), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, NY.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 97–159.

_____.1951. The genetical structure of populations. Ann. Eugenics 15: 323–354.

CONSEQUENCES OF VARIATION IN POLLEN LOAD SIZE[1]

A. G. Stephenson[2], M. R. Quesada[2], C. D. Schlichting[3], and J. A. Winsor[4]

ABSTRACT. As a consequence of relying on external agents to mediate the transfer of pollen, the flowers on a plant typically differ in the size of the pollen loads that are deposited onto their stigmas and in the number and identity of the plants that donate the pollen grains. These differences in the size and identity of the pollen loads, in turn, lead to differences in seed number per fruit, which lead to differences in the probability of fruit maturation and, ultimately, to differences in the vigor of the resulting progeny. We explore these interrelationships using our studies of *Cucurbita pepo* as an entry into the literature on these topics.

The remarkable variety of colors, shapes, and odors of flowers can be attributed to a large extent to the coevolution of plants and their myriad pollinators (Faegri and van der Pijl, 1979). No matter which agents transfer the pollen from the male to the female parts of flowers, however, typically there are variations in the genetic identity and number of pollen grains deposited onto the stigmas of an individual plant (e.g., Bertin, 1982a; Snow, 1982, 1986; Casper, 1983; McDade, 1983: Mulcahy et al., 1983; Ellstrand, 1984; McDade and Davidar, 1984; Garwood and Horvitz, 1985; Ganeshaiah et al., 1986; Barrett and Wolfe, 1986; Cruzan et al., 1988; Levin, 1990; Murcia, 1990: Uma Shaanker and Ganeshaiah, 1990; Spira et al., 1992). In this paper, we review the consequences of variation in the genetic identity and size of the pollen load deposited onto stigmas. In particular, we use our studies of the common zucchini (*Cucurbita pepo* cv. Black Beauty Bush, Cucurbitaceae) to illustrate the interrelationships among the size of the pollen load, seed number per fruit, the probability of fruit maturation, and progeny vigor. We then use our data as a starting point for discussion of the relevant literature.

DESCRIPTION OF *CUCURBITA PEPO* AND THE STUDY SITE

The Black Beauty Bush cultivar of zucchini is a monoecious, annual, short-internode vine with indeterminate growth and reproduction. The large, yellow flowers are borne individually and are bee-pollinated; each remains open for only a few hours on one morning. The mature fruit is cylindric in shape, has a volume of approximately three liters, and contains about 300 viable seeds each weighing 0.10–0.24 g. All of the field experiments described in this paper were performed at The Pennsylvania State University Agricultural Experiment Station at Rock Springs in Centre County, Pennsylvania.

POLLEN LOAD SIZE, SEED NUMBER, AND FRUIT MATURATION

Our studies of the consequences of variation in the size of the pollen loads deposited onto stigmas began in the summer of 1984 when we grew 60 zucchini plants at 2 m intervals at the field site at Rock Springs, PA (see Winsor et al., 1987). On 15 plants, every pistillate

[1]We thank Robert Oberheim, his staff, and the Department of Horticulture for use of the Agricultural Experiment Station. This work was supported by a Fulbright-Hays Fellowship and a Henry W. Popp Fellowship to MRQ, and NSF Grants BSR-8315612 A01, BSR-8818184, and DEB 91-09270 to AGS and JAW.

[2]Department of Biology, The Pennsylvania State University, University Park, PA 16802, U.S.A.
[3]Department of Ecology and Evolutionary Biology, U-43, University of Connecticut, Storrs, CT 06269, U.S.A.
[4]Department of Biology, The Pennsylvania State University, Altoona, PA 16603, U.S.A.

(female) flower received a small pollen load (240 + 36 grains [$\overline{X}$ + SD], N = 10). On another 15 plants, all pistillate flowers received a medium pollen load (2 × small pollen load). On a third group of 15 plants, all pistillate flowers received a large pollen load treatment in which the stigmas were saturated with pollen (>10,000 grains). On the final 15 plants, small, medium, and large pollen loads were alternated on the successive pistillate flowers produced on each plant. These four groups of 15 plants are termed the small, medium, large, and alternate plants (see Winsor et al., 1987; Stephenson et al., 1988a).

For the small, medium, and large pollen load plants, we found (a) that the size of the pollen load had a significant effect on seed number per fruit, (b) that the small and medium pollen load plants produced significantly more pistillate flowers than the large pollen load plants, but (c) that approximately 50% of the pistillate flowers produced a mature fruit (and 50% aborted early in development) in each of the three treatments. In short, when the size of the pollen load is varied *between* plants (the small, medium, and large pollen load plants), the probability that a pistillate flower will produce a mature fruit is independent of the size of the pollen load ($X^2 = 0.31$; df = 2; $p > 0.35$). In contrast, on the alternate pollen load plants, the probability that a flower would produce a mature fruit was not independent of the size of the pollen load ($X^2 = 40.6$; df = 2; $p < 0.0001$). The flowers receiving large pollen loads were far more likely to produce a mature fruit (71% maturation) than were the flowers receiving medium (28%) or small (18%) pollen loads (Winsor et al., 1987).

As with most outcrossing species, zucchini regularly produces far more flowers than mature fruits (see Lloyd, 1980; Stephenson, 1981; Sutherland and Delph, 1984; Lee, 1988). Hand-pollination studies, resource enrichment, and deprivation studies, as well as other types of evidence, indicate that outcrossing species are physiologically incapable of providing the necessary resources to develop mature fruits from all of their flowers (Lloyd, 1980; Stephenson, 1981; Lee, 1988). That is, an upper limit to fruit production is set by resources rather than flower number. When the number of pollinated flowers exceeds the resources available for fruit maturation (as commonly occurs), immature fruits abort until fruit production matches resource availability (Lloyd, 1980; Stephenson, 1981; Lee, 1988). Moreover, there is evidence from several species that fruit abortion is non-random with respect to seed number (e.g., Heinicke, 1917; Murneek, 1954; Bertin, 1982a; Lee and Bazzaz, 1982; Sarvas, 1962, 1968; Bawa and Webb, 1984; Ganeshaiah et al., 1986; Stephenson and Winsor, 1986a). Typically, a fruit with a below average number of seeds is more likely to abort than a fruit with a larger number of seeds.

In the zucchini study (Winsor et al., 1987), differences in seed number per fruit (and hence differences in the probability of abortion) are clearly related to differences in the number of pollen grains deposited onto stigmas. However, it is also possible that the identity of the pollen donors (rather than pollen load size per se) can also influence seed number. For example, it has long been known that selfing and biparental inbreeding will result in fruits with fewer seeds than those produced by cross-pollination (e.g., Darwin, 1877; Solbrig, 1976) due to partial self-incompatibility, genetic load, and other forms of inbreeding depression, late acting self-incompatibility, and post-zygotic self-incompatibility (e.g., Crowe, 1971; de Nettancourt, 1977; Seavey and Bawa, 1986; Bertin et al., 1989). Recently, a few studies have shown that fruits resulting from self-pollination not only contain fewer seeds, but they are also more likely to abort when fruits from both self- and cross-pollinations are present on the same inflorescence (Stephenson and Winsor, 1986b; Krebs and Hancock, 1990; Vaughton and Carthew, in press).

For example, in the partially self-incompatible plant *Lotus corniculatus* (Leguminosae), Stephenson and Winsor (1986b) selected three shoots on each of 20 plants. All of the flowers on one shoot from each plant were outcrossed while all of the flowers on a

second shoot were self-pollinated. On the third shoot, half of the flowers on each inflorescence were outcrossed and half were selfed. It was found that 8% of the flowers on the "selfed shoots" produced mature fruit while 35% of the flowers on "outcrossed shoots" produced mature fruit. Moreover, the outcrossed fruits contained significantly more mature seeds than the selfed fruits. On the "half shoots," however, 55% of the outcrossed flowers produced a mature fruit while only 1.4% of the selfed flowers produced a mature fruit (a significantly lower proportion than on the "selfed shoots"). In short, selfed flowers are more likely to produce a mature fruit when they are competing against other selfed flowers (also with below average seed numbers) than when they are competing against outcrossed fruits with higher seed numbers. Finally, there are data indicating that *Campsis radicans* and *Asclepias speciosa* can also discriminate among fruits in which the seeds have been sired by different non-self pollen donors (Bertin, 1982b, 1985; Bookman, 1984). In each of these cases the "favored" donors produced more seeds per fruit than the "non-favored" donors.

Together, these studies indicate that the effects of variations in pollen load size and sire identity on fruit abortion are related to differences in the number of resulting seeds. This relationship is reasonable in light of what is known about the physiology of fruit and seed development. For example, in most species there is little or no growth of the ovary during anthesis (Crane, 1964; Coombe, 1976). The deposition of pollen, which is a rich source of hormones (auxins, gibberellins, brassinolides), on the stigmatic surface provides the stimulus for the renewed growth of the ovary (e.g., Nitsch, 1952; Bollard, 1970; Vasil, 1974). After fertilization, the developing seeds produce hormones that establish and regulate the source-sink interactions that govern fruit development (e.g., Bollard, 1970; Brenner, 1987; Bangerth, 1989). It is, therefore, possible that differences in pollen deposition and seed number lead to differences in hormone levels in the developing fruit.

Two recent studies, however, have uncoupled the effects of pollen load size and sire identity on fruit abortion from the effects of seed number. In the dioecious shrub *Lindera benzoin*, fruit abortion among pollinated flowers is non-random with respect to the number of pollen tubes growing in the styles of the uniovulate flowers (Niesenbaum and Casper, 1994). In *Phormium tenax*, selfed fruits are far more likely to abort on inflorescences containing mixtures of self- and cross-pollinated flowers, even though selfed fruits on selfed only inflorescences produced as many seeds as cross-pollinated flowers on other inflorescences (Becerra and Lloyd, 1992). The seeds from self- and cross-pollinations were not only similar in number but were also similar in weight, prompting Becerra and Lloyd to hypothesize that hormonal signals from the growing pollen tubes alone are sufficient in this species to result in non-random abortion/maturation of self- and cross-pollinated fruits.

When variations in the size of the pollen load or when variations in the identity of the donors lead to differences in seed number per fruit, the abortion of fruits on the basis of seed number typically results in an economy of seed packaging (e.g., Mitchell, 1977; Lee and Bazzaz, 1982; Bookman, 1984; Ganeshaiah et al., 1986; Stephenson, 1992). For example, in *Lotus corniculatus*, which normally aborts about 50% of its immature fruits, a regression of dry weight of the pericarp per seed on the number of seeds per fruit is highly significant ($r^2 = -0.57$; $N = 184$; $p < 0.0001$) (Stephenson and Winsor, 1986a). Moreover, because fruits tend to abort when they are small (see Stephenson, 1981), the amount of resources saved by the more efficient packaging of seeds is sufficient to defray most of the cost of aborting an immature fruit. For example, Bookman (1984) found the average aborted capsule of *Asclepias speciosa* contained about 113 seeds, whereas the average mature fruit contained about 170 seeds. Her regression analysis revealed that mature fruits with 113 seeds require about 2.85 mg more pericarp per seed than do mature fruits with 170 seeds (a savings in packaging of 162.5 mg). Meanwhile, most of the aborted fruits weighed 3 mg or less and the

dry weight of an entire flower is only 33–43 mg, including pollinia, nectar, etc. (Bookman, 1983). All else being equal, the improvement in packaging efficiency alone could support the production of four additional flowers. In short, the resource efficiency gained by aborting fruits on the basis of seed number may defray much of the cost of producing surplus flowers.

EFFECT OF POLLEN LOAD SIZE ON PROGENY VIGOR

In order to determine if variations in the sizes of the pollen loads deposited onto stigmas have an effect on the vigor of the resulting progeny, we performed a series of experiments using the seeds from the "large" and "small" pollen load plants described above (Winsor et al., 1987). Seeds from large and small pollen load plants were germinated and grown in a greenhouse, and various measures of vigor were taken ranging from days to seedling emergence to dry weight at 30 days after emergence. We found that the size of the pollen load had a significant effect on nearly all of our measures of vigor (days to emergence, stem circumference, leaf area at 20 and 30 days, and dry weight at 30 days after emergence). The progeny from the large pollen loads were more vigorous than those from small pollen loads (Winsor et al., 1987).

We also performed a similar experiment with the seeds from the small and large pollen load fruits from the "alternate" pollen load plants (Stephenson et al., 1986). Again, we found that the progeny from large pollen loads outperformed the progeny from small pollen loads. In order to determine if the results from the greenhouse studies of progeny vigor could be extended to the field, we planted 500 seeds from the small and large pollen load plants in an experimental garden at the field site and monitored germination of all seeds and flower, fruit and seed production from a sample of 92 plants. We found that the progeny from the large pollen loads were not only more likely to germinate, but they also had significantly greater reproductive output than the plants sired by small pollen loads (Davis et al., 1987). Finally, a greenhouse study revealed that after two years of storage, the differences in seed germination percentage and speed of germination were even greater between the seeds produced by large and small pollen loads (unpubl. data). In summary, it appears that zucchini improves the average quality of its offspring by aborting fruits on the basis of seed number.

In two other species it has also been shown that the non-random abortion of fruits can improve offspring quality. In *Lotus corniculatus*, the seeds obtained by random patterns of abortion (accomplished by hand-thinning inflorescences prior to any natural fruit abortion) are less likely to germinate, grow more slowly under greenhouse conditions, and have reduced reproductive output under field conditions compared to seeds from inflorescences in which the fruits were allowed to abort on the basis of seed number (Stephenson and Winsor, 1986a; Stephenson et al., 1988b). Recently, Bertin and Peters (1992) have shown that the non-random abortion of fruits on the basis of donor identity tends to enhance progeny performance in *Campsis radicans*. Previous studies had shown that the "preferred donors" tend to produce fruits containing more mature seeds (Bertin, 1982b, 1985).

In the zucchini studies, the effects of non-random fruit abortion on progeny vigor are associated with differences in the size of the pollen load that was deposited onto the stigmas. In addition to the zucchini studies, several studies over the past two decades have also shown a relationship between the number of pollen grains deposited onto a stigma and the performance of the resulting offspring (see Mulcahy and Mulcahy, 1987). In general, these studies have shown that the progeny produced by large pollen loads have enhanced germination, more vigorous vegetative growth, and/or greater reproductive output than the progeny from smaller pollen loads (e.g., Mulcahy et al., 1975, 1978; Fingerett, 1979; Lee and Hartgerinck, 1986; McKenna, 1986; Ramstetter and Mulcahy, 1988; Bertin, 1990; Richardson and Stephenson, 1992; but see Snow, 1990). The causes of these effects of pollen load size on

progeny vigor have been the subject of much debate (Mulcahy, 1979; Charlesworth, 1988; Stephenson et al., 1988c; Snow and Mazer, 1988; Lyons et al., 1989; Schlichting et al., 1990) and both pre- and post-zygotic hypotheses have been advanced as explanations.

Mulcahy (1979) was the first to formulate a hypothesis for the effect of pollen load size on progeny performance. He argued that under conditions of pollen competition (i.e., more pollen tubes than ovules) only the fastest-growing pollen tubes achieve fertilization, whereas both fast- and slow-growing pollen tubes achieve fertilization when there are fewer pollen tubes than ovules. According to this pollen competition hypothesis (PCH), the correlation between fast-growing pollen tubes and vigorous progeny is due to an overlap in gene expression between the two stages of the life cycle.

When the PCH was formulated, it was assumed that pollen competition could only act through selection on heritable (additive) genetic variation in pollen performance among the microgametophytes deposited onto the stigma. However, genetically based pollen-pistil interactions leading to differences in pollen performance could also account for the effects of large pollen loads on progeny vigor, if the differences are determined by genetic similarity/dissimilarity between the maternal sporophyte and the microgametophyte (see Waser et al., 1987; Snow and Mazer, 1988; Schlichting et al., 1990). This non-additive genetic variation could be maintained in a population because the relative performance of pollen of different genotypes would vary with the recipient plant (Stephenson and Bertin, 1983; Waser et al., 1987; Schlichting and Devlin, 1989; Bertin, 1990).

Alternatively, because fruits produced from large pollen loads typically differ from those produced by small pollen loads in seed number, in the position of the seeds within the fruit (position of the fertilized ovules), and in seed weight, it has been suggested that any differences in progeny vigor could be due to differences in the quantity or quality of the resources provided to the seeds by the maternal parent (Charlesworth, 1988; Schlichting et al., 1990). This hypothesis attributes differences in progeny performance to post-zygotic maternal effects.

Finally, because most species regularly produce more ovules than mature seeds (see Lee, 1988), differences in progeny vigor related to pollen load size could be due to non-random seed abortion. This hypothesis argues that if differences in vigor among the developing seeds within a fruit influence which seeds mature, as indicated by at least two studies (Casper, 1988; Rocha and Stephenson, 1991), then fruits produced by large pollen loads would have more fertilized ovules and would have greater opportunities for post-zygotic competition/non-random seed abortion.

TESTS OF THE POLLEN COMPETITION HYPOTHESIS

Beginning in 1985, we initiated a series of experiments designed to critically examine the PCH. This hypothesis not only predicts that the offspring derived from large pollen loads should be more vigorous than offspring from small pollen loads, it also predicts that the offspring from large loads should be less variable in their performance due to truncation selection (see Mulcahy, 1979; Schlichting et al., 1987). Consequently, we carefully re-examined the greenhouse data on vigor obtained from the progeny of large and small pollen loads (see above; Winsor et al., 1987) and found that the performance of the progeny from the large pollen load plants is significantly less variable than that of the progeny from small pollen loads for each trait that differed significantly in mean performance (see Schlichting et al., 1987). In short, the progeny from large pollen loads not only grow faster but they are more uniform in their growth.

We also performed a series of studies to determine if the seeds resulting from small and large pollen loads using the Black Beauty cultivar of zucchini were due to additive

genetic variance in the genes expressed during both stages of the life cycle (Schlichting et al., 1990). We first produced, via mass selection, seeds that were derived from two generations of large pollen loads (LL seeds), two generations of small pollen loads (SS seeds), and LS seeds and SL seeds. Two studies examined the transmission of the pollen load effect to subsequent generations through the ovules (female role). A third study determined if there is heritable genetic variation for pollen performance and if the pollen load effect could be transmitted to a subsequent generation through the pollen (male role). In each of these studies the vigor of the progeny from the subsequent generations was evaluated in the greenhouse and/or the field.

The results of these studies (Schlichting et al., 1990) revealed (1) that pollen performance (the ability to sire seeds) does respond to selection imposed by large pollen loads, (2) that 23 of the 35 total traits of the progeny that we measured in the three studies of transmission to subsequent generations changed in the direction predicted by the heritable variation hypothesis, (3) that 5 of the 35 traits were significantly affected by the size of the pollen load that produced the previous generation (all five were in the direction predicted by the PCH), and (4) that one study produced an overall significant difference (MANOVA) attributable to the size of the pollen load that produced the previous generation (it too was in the direction predicted by the PCH).

From these experiments we concluded that additive genetic variance in genes expressed during both stages of the life cycle plays a real but minor role in the production of differences in vigor between progeny arising from small versus large pollen loads in the Black Beauty bush cv. of zucchini (Schlichting et al., 1990). Perhaps pollen-pistil interactions (non-additive genetic variation), maternal effects (although we controlled for seed size), and/or non-random patterns of seed abortion play the more important roles. However, these results were not completely unexpected because there is little genetic variation in the Black Beauty cultivar.

Consequently, we wanted to determine if the PCH is more important in plants with greater genetic variation (see Quesada et al., 1993). We first produced an F_1 hybrid of the common zucchini with its wild progenitor. This hybrid had similar levels of heterozygosity to natural populations of outcrossing herbaceous dicots. We again experimentally varied the intensity of pollen competition by depositing either large or small pollen loads onto stigmas. In order to separate the effects of pollen competition from maternal effects associated with differences in seed number and weight, we excised the styles of flowers receiving large pollen loads after only the fastest pollen tubes had entered the ovary. In contrast, the styles from the flowers receiving the small pollen loads were excised after both the fast and the slow-growing tubes had entered the ovary. Consequently, the mature fruits from the two pollen load treatments contained similar numbers and sizes of seeds despite great differences in the intensity of pollen competition to sire those seeds. Moreover, there are no *a priori* differences between the two pollination treatments in the opportunity for non-random seed abortion.

Previous studies (Stephenson et al., 1988d) had revealed that fast- and slow-growing pollen tubes fertilize ovules in different regions (locations) within the ovary. Fast-growing tubes fertilize ovules in the stylar end of the ovary (region 1), while slower growing tubes fertilize ovules in the peduncular end of the ovary (region 3). Consequently, we examined the vigor of the progeny produced in regions 1 and 3 from both large and small pollen loads.

Quesada et al. (1993) found that the progeny produced from large pollen loads significantly outperform the progeny from small pollen loads. We also found a significant interaction between the size of the pollen load and the region of the fruit where the seeds were produced. For small pollen loads, the progeny from the stylar region of the ovary (region 1) outperform the progeny from the peduncular region (region 3). These findings indicate that the progeny from the fastest pollen tubes outperform the progeny from the slowest tubes.

Moreover, these differences in progeny vigor are unlikely to be due to maternal effects or non-random seed abortion. Experiments are currently underway to determine how much of this variation in pollen performance is heritable.

Together, these tests indicate that the effects of pollen load size are due, at least in part, to genetic variation in pollen tube growth rates that result in non-random fertilization. Moreover, it appears that the effects of pollen competition on progeny performance increase with the genetic variation present in the pollen producing sporophyte. These findings are supported by a recent study showing that zucchini pollen of different genotypes differ in their growth rates *in vivo* and in their ability to achieve fertilization when mixtures are applied to stigmas (Quesada et al., 1991). In addition, Snow and Spira (1991a, b) have recently shown that the growth rates of pollen from different *Hibiscus moscheutus* plants grown in experimental gardens often differ dramatically, and that these differences are maintained across different female recipients. Although it will be necessary to obtain data from many more species, especially from natural populations, clearly there is potential for non-random fertilization in flowers that receive large pollen loads and that non-random fertilization can affect progeny performance and be passed on to subsequent generations.

CONCLUSIONS AND SUMMARY

Because plants rely on external agents to mediate pollen transfer, they have little direct control over either the number or the genotypes of the pollen grains that are deposited onto their stigmas. In contrast to the haphazard nature of pollen deposition, however, this paper has reviewed the evidence showing that events following pollination are often non-random with respect to (1) which genotypes of the deposited pollen actually fertilize the ovules (slow-growing pollen tubes are less likely to achieve fertilization, particularly under conditions of pollen competition), (2) which genotypes of the seeds mature (e.g., selfed seeds and seeds sired by close relatives are more likely to abort; see also Casper, 1988; Rocha and Stephenson, 1991), and (3) which fruits mature/abort. Often fruits sired by small pollen loads and those containing less than a full complement of seeds are the most likely to abort. Moreover, many studies have shown that the size of the pollen load has a significant affect on the performance of the resulting progeny, and recent studies have shown that this is due, at least in part, to non-random fertilization by vigorous pollen genotypes under conditions of pollen competition, as first suggested by Mulcahy (1979). Consequently, by aborting fruits on the basis of seed number, plants eliminate those fruits produced under conditions of little or no pollen competition, as well as those fruits produced by self-pollination and matings with close relatives, and thereby improve the average quality of their seed crops.

Finally, as Lee (1988) adumbrated, the non-random events from pollen deposition to fruit maturation are the consequence of known (or knowable) genetic and physiological mechanisms, such as the source-sink interactions that govern all growth processes in plants, including seed maturation and fruit growth. This is not to say that there remain no issues of debate. Quite to the contrary, one active area of debate and research concerns the search for mechanisms that maintain genetic variation for pollen performance within a population (e.g., Charlesworth and Charlesworth, 1992). It should also be noted that the recognition of the non-random events from pollen deposition to fruit maturation and their potential effects on progeny performance are not new with this paper. Bertin (1982b) and later Stephenson and Bertin (1983) referred to these events as an "integrated genetic sieve," while Stearns (1987) called them "selection arenas," but even these authors must trace the origin of these ideas to Darwin (1877), Buchholz (1918), and Correns (1914).

LITERATURE CITED

Bangerth, F. 1989. Dominance among fruits/sinks and the search for a correlative signal. Physiol. Plantarum 76: 608–614.

Barrett, S. C. H. and L. M. Wolfe. 1986. Pollen heteromorphism as a tool in studies of the pollination process in *Pontederia cordata* L. Pp. 435–442 *in* D. L. Mulcahy, G. B. Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, New York.

Bawa, K. S. and C. J. Webb. 1984. Flower, fruit, and seed abortion in tropical trees: implications for the evolution of paternal and maternal reproductive patterns. Amer. J. Bot. 71: 736–751.

Becerra, J. X. and D. G. Lloyd. 1992. Competition dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): a second action of self-incompatibility at the whole flower level? Evolution 46: 458–469.

Bertin, R. I. 1982a. Floral biology, hummingbird pollination, and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). Amer. J. Bot. 69: 122–134.

_____. 1982b. Paternity and fruit production in trumpet creeper (*Campsis radicans*). Amer. Naturalist 119: 694–709.

_____. 1985. Non-random fruit production in Campsis radicans: between-years consistency and effects of prior pollinations. Amer. Naturalist 126: 750–759.

_____. 1990. Effects of pollination intensity in *Campsis radicans*. Amer. J. Bot. 77: 178–187.

_____, C. Barnes, and S. I. Guttman. 1989. Self-sterility and cryptic self-fertility in *Campsis radicans* (Bignoniaceae). Bot. Gaz. 150: 397–403.

_____ and P. J. Peters. 1992. Paternal effects on offspring quality in *Campsis radicans*. Amer. Naturalist 140: 166–178.

Bollard, E. G. 1970. The physiology and nutrition of developing fruits. Pp. 387–427 *in* A. C. Hulme (ed.), The Biochemistry of Fruits and Their Products. Vol. 1. Academic Press, New York.

Bookman, S. S. 1983. Costs and benefits of flower abscission and fruit abortion in *Asclepias speciosa*. Ecology 64: 264–273.

_____. 1984. A demonstration of sexual selection in plants (*Asclepias speciosa* Torr.). Evolution 38: 72–83.

Brenner, M. L. 1987. The role of hormones in photosynthate partitioning and seed filling. Pp. 474–493 *in* D. J. Davies (ed.), Plant Hormones and Their Role in Plant Growth and Development. M. N. Shoff, Minneapolis, MN.

Buchholz. J. T. 1918. Suspensor and early embryo of *Pinus*. Bot. Gaz. 66: 185–228.

Casper, B. B. 1983. The efficiency of pollen transfer and rates of embryo initiation in *Cryptantha* (Boraginaceae). Oecologia 59: 262–268.

_____. 1988. Evidence for selective embryo abortion in *Crypantha flava*. Amer. Naturalist 132: 318–326.

Charlesworth, D. 1988. A comment on the evidence for pollen competition in plants and its relationship to progeny fitness. Amer. Naturalist 132: 298–302.

_____ and B. Charlesworth. 1992. The effects of selection in the gametophytic stage on mutational load. Evolution 46: 703–720.

Coombe, B. G. 1976. The development of fleshy fruits. Ann. Rev. Pl. Physiol. 27: 507–528.

Correns, C. 1914. Die Bestimmung und Vererbung des Geschlechtes: nach neuen Versuchen mit höheren Pflanzen. Bornträger, Berlin.

Crane, J. C. 1964. Growth substances in fruit setting and development. Ann. Rev. Pl. Physiol. 15: 303–326.

Crowe, L. K. 1971. The polygenic control of outbreeding on *Borago officinalis*. Heredity 27: 111–118.

Cruzan, M. B., P. R. Neal, and M. F. Willson. 1988. Floral display in *Phyla incisa*: consequences for male and female reproductive success. Evolution 42: 505–515.

Darwin, C. 1877. The effects of cross- and self-fertilisation in the vegetable kingdom. D. Appleton, London.

Davis, L. E., A. G. Stephenson, and J. A. Winsor. 1987. Pollen competition improves performance and reproductive output of the common zucchini squash under field conditions. J. Amer. Soc. Hort. Sci. 112: 711–716.

Ellstrand, N. C. 1984. Multiple paternity within the fruits of the wild radish, *Raphanus sativus*. Amer. Naturalist 123: 819–828.

Faegri, D. and L. van der Pijl. 1979. The Principles of Pollination Ecology, Third revised edition. Pergamon Press, Oxford.

Ganeshaiah, K. N., R. Uma Shaanker, and G. Shivashanker. 1986. Stigmatic inhibition of pollen grain germination—its implications for frequency distribution of seed number in pods of *Leucaena leucocephala* (Lam.) de Wit. Oecologia 70: 568–572.

Garwood, N. C. and C. C. Horvitz. 1985. Factors limiting fruit and seed production of a temperate shrub, *Staphylea trifolia* L. (Staphyleaceae). Amer. J. Bot. 72: 453–466.

Heinicke, A. J. 1917. Factors influencing the abscission of flowers and partially developed fruits of the apple (*Pyrus Malus* L.). Cornell Univ. Agric. Exp. Sta. Bull. 393: 43–114.

Krebs, S. L. and J. F. Hancock. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L. Theor. Appl. Genet. 79: 825–832.

Lee, T. D. 1988. Patterns of fruit and seed production. Pp. 179–202 *in* J. Lovett Doust and L. Lovett Doust (eds.), Plant Reproductive Ecology: Patterns and Strategies. Oxford Univ. Press, New York.

_____ and F. A. Bazzaz. 1982. Regulation of fruit maturation pattern in an annual legume: *Cassia fasciculata*. Ecology 63: 1374–1388.

_____ and A. P. Hartgerinck. 1986. Pollination intensity, fruit maturation pattern, and offspring quality in *Cassia fasciculata* (Leguminosae). Pp 417–422 *in* D. L. Mulcahy, G. B. Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, New York.

Levin, D. A. 1990. Sizes of natural microgametophyte populations in pistils of *Phlox drummondii*. Amer. J. Bot. 77: 356–363.

Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytol. 86: 69–80.

Lyons, E. E., N. M. Waser, M. V. Price, J. Antonovics, and A. F. Motten. 1989. Sources of variation in plant reproductive success and implications for concepts of sexual selection. Amer. Naturalist 134: 409–433.

McDade, L. A. 1983. Pollination intensity and seed set in *Trichanthera gigantea* (Acanthaceae). Biotropica 15: 122–124.

_____ and P. Davidar. 1984. Determinants of fruit and seed set in *Pavonia dasypetala* (Malvaceae). Oecologia 64: 61–67.

McKenna, M. A. 1986. Heterostyly and microgametophytic selection: the effect of pollen competition on sporophyte vigor in two distylous species. Pp. 443–448 *in* D. L. Mulcahy, G. B. Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, New York.

Mitchell, R. 1977. Bruchid beetles and seed packaging by palo verde. Ecology 58: 644–651.

Mulchay, D. L. 1979. The rise of the angiosperms: a genecological factor. Science 206: 20–23.

_____, P. S. Curtis, and A. A. Snow. 1983. Pollen competition in a natural population. Pp. 330–337 *in* C. E. Jones and R. J. Little (eds.), Handbook of Pollination Biology. Academic Press, New York.

_____ and G. B. Mulchay. 1987. The effects of pollen competition. Amer. Scientist 75: 44–50.

_____, _____, and E. Ottaviano. 1975. Sporophytic expression of gametophytic competition in *Petunia hybrida*. Pp. 227–232 *in* D. L. Mulchay (ed.), Gamete Competition in Plants and Animals. North Holland Publishing Company, Amsterdam.

_____, _____, and _____. 1978. Further evidence that gametophytic selection modifies the genetic quality of the sporophyte. Societé Botanique Française, Actualités Botaniques 1–2: 57–60.

Murcia, C. 1990. Effect of floral morphology and temperature on pollen receipt and removal in *Ipomoea trichocarpa*. Ecology 71: 1098–1109.

Murneek, A. E. 1954. The embryo and endosperm in relation to fruit development; with special reference to the apple, *Malus sylvestris*. Proc. Amer. Soc. Hort. Sci. 64: 573–582.

Nettancourt, D. de. 1977. Incompatibility in Angiosperms. Springer-Verlag, Berlin and New York.

Niesenbaum, R. A. and B. B. Casper. 1994. Pollen tube numbers and selective fruit maturation in *Lindera benzoin*. Amer. Naturalist 144: 184–191.

Nitsch, J. P. 1952. Plant hormones and the development of fruits. Quart. Rev. Biol. 27: 33–57.

Quesada, M., C. D. Schlichting, J. A. Winsor, and A. G. Stephenson. 1991. Effects of genotype on pollen performance in *Cucurbita pepo*. Sexual Pl. Reprod. 4: 208–214.

_____, J. A. Winsor, and A. G. Stephenson. 1993. Effects of pollen competition on progeny performance in a heterozygous cucurbit. Amer. Naturalist 142: 694–706.

Ramstetter, J. and D. L. Mulcahy. 1988. Consequences of pollen competition for *Aureolaria flava* seedlings. Bull. Ecol. Soc. Amer. Suppl. 69: 269–270.

Richardson, T. E. and A. G. Stephenson. 1992. Effects of parentage and size of the pollen load on progeny performance in *Campanula americana*. Evolution 46: 1731–1739

Rocha, O. J. and A. G. Stephenson. 1991. Effects of nonrandom seed abortion on progeny performance in *Phaseolus coccineus* L. Evolution 45: 1198–1208.

Sarvas, R. 1962. Investigations on the flowering and seed crop of *Pinus sylvestris*. Commun. Inst. For. Fenn. 53: 1–198.

_____. 1968. Investigations on the flowering and seed crop of *Picea abies*. Commun. Inst. For. Fenn. 67: 1–84.

Schlichting, C. D. and B. Devlin. 1989. Male versus female reproduction success in the hermaphroditic plant *Phlox drummondii*. Amer. Naturalist 133: 212–227.

_____, A. G. Stephenson, L. E. Davis, and J. A. Winsor. 1987. Pollen competition and offspring variance. Evol. Trends Pl. 1: 35–39.

_____, A. G. Stephenson, L. E. Small, and J. A. Winsor. 1990. Pollen loads and progeny vigor in *Cucurbita pepo*: The next generation. Evolution 44: 1358–1372.

Seavey, S. R. and K. S. Bawa. 1986. Late acting self-incompatibility in angiosperms. Bot. Rev. 52: 195–219.

Snow, A. A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. Oecologia 55: 231–237.

_____. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. Amer. J. Bot. 73: 139–151.

_____. 1990. Effects of pollen-load size and number of donors on sporophyte fitness in wild radish (*Raphanus raphanistrum*). Amer. Naturalist 136: 742–758.

_____ and S. J. Mazer. 1988. Gametophytic selection in *Raphanus raphanistrum*: A test for heritable variation in pollen competitive ability. Evolution 42: 1065–1075.

_____ and J. P. Spira. 1991. Pollen vigor and the potential for sexual selection in plants. Nature 352: 796–797.

_____ and _____ . 1991b. Differential pollen-tube growth rates and nonrandom fertilization in *Hibiscus moscheutos* (Malvaceae). Amer. J. Bot. 78: 1419–1426.

Solbrig, O. T. 1976. On the relative advantages of cross- and self-fertilization. Ann. Missouri Bot. Gard. 63: 262–276.

Spira, T. P., A. A. Snow, D. G. Whigham, and J. Leak. 1992. Flower visitation, pollen deposition, and pollen-tube competition in *Hibiscus moscheutos* (Malvaceae). Amer. J. Bot. 79: 428–433.

Stearns, S. C. 1987. The selection arena hypothesis. Pp. 337–349 *in* S. C. Stearns (ed.), The Evolution of Sex and its Consequences. Birkhauser Verlag, Basel.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Ann. Rev. Ecol. Syst. 12: 253–279.

_____. 1992. The regulation of maternal investment in plants. Pp. 151–171 *in* C. Marshall and J. Grace (eds.), Environmental Physiology and Ecology of Fruit and Seed Production. Cambridge Univ. Press, Cambridge.

_____ and R. I. Bertin. 1983. Sexual selection in plants. Pp. 109–150 *in* L. Real (ed.), Pollination Biology. Academic Press, New York.

_____, B. Devlin, and J. B. Horton. 1988a. The effects of seed number and prior fruit dominance on the pattern of fruit production in *Cucurbita pepo* (Zucchini Squash). Ann. Bot. 62: 653–661.

_____, R. S. Johnson, and J. A. Winsor. 1988b. Performance of *Lotus corniculatus* L. produced by random and natural patterns of fruit abortion when grown under competitive conditions. Amer. Midl. Naturalist 120: 102–107.

_____ and J. A. Winsor. 1986a. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. Evolution 40: 453–458.

_____ and _____ . 1986b. *Lotus corniculatus* selectively aborts fruits resulting from self-pollination. Lotus Newslett. 16: 9–10.

_____, _____, and L. E. Davis. 1986. Effects of pollen load size on fruit maturation and sporophyte quality in zucchini. Pp. 429–434 *in* D. L. Mulcahy, G. B. Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, New York.

_____, _____, and C. D. Schlichting. 1988d. Evidence for non-random fertilization in the common zucchini, *Cucurbita pepo*. Pp. 333–338 *in* M. Cresti, P. Gori, and E. Pacini (eds.), Sexual Reproduction in Higher Plants. Springer-Verlag, Berlin.

_____, _____, _____, and L. E. Davis. 1988c. Pollen competition, nonrandom fertilization, and progeny fitness: a reply to Charlesworth. Amer. Naturalist 132: 303–308.

Sutherland, S. and L. F. Delph. 1984. On the importance of male fitness in plants: patterns of fruit set. Ecology 65: 1093–1104.

Uma Shaanker, R. and K. N. Ganeshaiah. 1990. Pollen grain deposition patterns and stigma strategies in regulating seed number per pod in multi-ovulated species. Pp. 165–178 *in* K. S. Bawa and M. Hadley (eds.), Reproductive Ecology of Forest Plants. Man and the Biosphere Series, Vol. 7. Academic Press, New York.

Vasil, J. K. 1974. The histology and physiology of pollen germination and pollen growth on the stigma and in the style. Pp. 105–118 *in* H. F. Linskens (ed.), Fertilization in Higher Plants. North Holland, Amsterdam.

Vaughton, G. and S. M. Carthew. In press. Evidence for selective fruit abortion in *Banksia spinulosa* (Proteaceae). Biol. J. Linn. Soc.

Waser, N. M., M. V. Price, A. M. Montalvo, and R. N. Gray. 1987. Female mate choice in a perennial herbaceous wildflower, *Delphinium nelsonii*. Evol. Trends Pl. 1: 29–33.

Winsor, J. A., L. E. Davis, and A. G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. Amer. Naturalist 129: 643–656.

REGULATION OF FLOWER, FRUIT AND SEED PRODUCTION: *PHASEOLUS COCCINEUS*, A STUDY CASE[1]

O. J. Rocha[2] and A. G. Stephenson[3]

ABSTRACT. In this paper, we first examine the factors that determine flower, fruit, and seed production in plants by summarizing our experiments with *Phaseolus coccineus*. We then place the results of our work in a broader context via a series of discussions following each experiment. We describe the patterns of flower, fruit, and seed production at two different levels: whole plant and within inflorescences. At the whole plant level, we found substantial variation in the number of flowers, fruits, and seeds produced by each plant, but in all plants flower production over time typically followed a sigmoidal pattern. Despite the large number of flowers (mean (S.E.) = 370.6 (± 17.5)) produced per plant, fruit production was very low (mean (S.E.) = 26.4 (± 1.2)). Moreover, only half of the ovules in those fruits produced seed. We conclude that the balance in resource allocation between growth and reproduction appears to be controlling the time of initiation and duration of flowering, as well as the number of flowers, fruits, and seeds produced.

Although sufficient pollen was deposited onto most flowers to insure complete fertilization of the ovules (over 59% of the flowers examined had more that 20 pollen tubes growing into their styles), most of these flowers did not set fruit. These findings indicate that pollen deposition did not limit fruit production in our experimental garden. In addition, manual cross pollination (using single donor or multiple donors) did not increase the proportion of fruits matured over that found in naturally pollinated flowers. However, manual self-pollination significantly reduced the proportion of fruits matured.

We found that within inflorescences, the presence of 8- to 15-day-old developing fruits significantly reduced subsequent flower and fruit production. The period when flower and fruit production declined coincided with the period of greatest size increase in the developing fruit. This finding suggests that the production of flowers and fruits is inhibited during the period of high demand for nutrients and carbohydrates by the developing fruits.

Seed maturation is not independent of the position of the ovule within the ovary. In general, the three ovule positions closest to the style are more likely to produce seeds than the three most basal ovule positions. Apparently, non-maturing ovules aborted after fertilization: even when only about 50% of the ovules matured into seeds, 98% of the ovules examined in 752 fruits had been fertilized.

The production of flowers, fruits, and seeds by flowering plants is influenced by two groups of factors. The first group includes environmental factors over which the plant has little or no control, such as pollen and pollinator availability, the rate and type of pollen

[1]We thank M.E. Zaldivar, M. Quesada, T.C. Lau, T.E. Richardson, B. Devlin, C. Schlichting, J.A. Winsor, and R. Marquis for commenting on a previous version of this manuscript. We also thank L. Hartzel, T. Omeis, C. Taylor, P. Traynor, J.A. Díaz, and K. Bogantes for greenhouse and field assistance. We thank R. Oberheim, his staff and the Department of Horticulture for the use of the PSU Agricultural Experimental Station at Rock Springs and E. Jimenez for the use of his farm at Los Sitios, Moravia, Costa Rica. This work was supported by a Fulbright-Hays Fellowship, a Henry W. Popp Fellowship, and UCR Grant 111-91-223 to O.J.R., and NSF Grants BSR83-1561A01, BSR88-18184, and BSR91-09270 to A.G.S.

[2]Escuela de Biología, Universidad de Costa Rica, Cuidad Universitaria "Rodrigo Facio," San José, Costa Rica, America Central.

[3]Department of Biology and Graduate Program in Ecology, The Pennsylvania State University, 208 Mueller Laboratory, University Park, Pennsylvania, 16802, U.S.A.

deposited onto the stigma, and environmental factors that affect the viability of pollen, the receptivity of the stigma, and the activity of the pollinators. This group also includes environmental factors that affect the acquisition of resources by the plant. The second group includes those factors that are governed by the genotype of the plant. Here we include the complex processes that lead to self-incompatibility and other less understood pollen-pistil interactions. This group also includes genetically determined characteristics that influence the ability of the plant to cope with environmental factors that affect its productivity and also traits that affect the way in which available resources are allocated to growth, survival, and reproduction.

In this paper, we review the factors that regulate flower, fruit, and seed production by describing some of our experiments with *Phaseolus coccineus*, conducted over the last six years both in the United States and in Costa Rica. We then relate our results to those of other studies via a series of discussions following each experiment. We address this topic at two levels: the patterns of flower, fruit, and seed production for the whole plant, and patterns of production within inflorescences.

EXPERIMENT 1: General patterns of flower, fruit, and seed production.

The scarlet runner bean, *Phaseolus coccineus* L. (Leguminosae), is native to mid-altitudes of southern Mexico and Central America. Most *P. coccineus* plants are viny perennials, but are cultivated throughout the world as an annual crop. The perfect flowers are self-incompatible, bee-pollinated, morphologically typical of the subfamily Papilionoideae, and are borne in long racemes of 12–20 flowers. The fruit is a legume. It requires 6 to 7 weeks to mature and is 10–30 cm long, 1.5–2.0 cm wide, with up to seven flattened, reniform, or almost cylindrical seeds, with smooth skin, 0.4–3.0 cm long, 0.3–1.4 cm wide, and 0.2–1.3 cm thick (Rocha and Stephenson, 1990, 1991).

In order to determine the general pattern of reproduction of this species, 200 total seeds from six plants were germinated in a greenhouse during the spring of 1989. Two weeks after emergence, 167 seedlings were transplanted to an experimental garden at The Pennsylvania State University Agricultural Station at Rock Springs, Pennsylvania. The plants were grown 1 m apart and randomly interspersed in a population of 68 additional non-experimental plants that served as sources of pollen. We monitored plants daily and recorded flower production. Flowers were permitted to be open-pollinated. As fruits matured, we harvested them and determined the number of seeds per fruit.

We found substantial variation in flower, fruit, and seed production in our experimental population (Table 1). A significant portion of the observed variation could be explained by the genotype of the maternal plant that produced the seeds and by the conditions in the ovary under which the seeds were produced (i.e., the number of seeds within the fruit and the position of the seed within the ovary (Rocha and Stephenson, 1990, 1991)). In spite of the large number of flowers (370 ± 17.5) produced per plant in the experimental population, fruit production was very low (Table 1). Our results showed that, on average, only 7.8% of the flowers set fruit. In addition, only about half of the ovules in those fruits produced seeds (Table 1).

Many plant species regularly abscise a large portion of their flowers and immature fruits (Lloyd, 1980; Stephenson, 1981, Sutherland and Delph, 1984; Lee, 1988). For example, others have shown that some varieties of *Phaseolus vulgaris* abscise 45–80% of their initiated fruits (Subhadrabandhu et al., 1978; Pechan and Webster, 1986, 1986b; Sage and Webster, 1987). Bawa and Webb (1984) described the patterns of flower, fruit, and seed production in seven tropical lowland forest trees. They found that only 1–26% of the flowers developed mature fruits. In the herbaceous legume *Cassia fasciculata* 24–70% of initiated fruits matured (Lee and Bazzaz, 1982, 1982b).

Table 1. Mean, standard error (S.E.) and range for flower, fruit and seed production for 167 plants grown in an experimental garden in 1989 at The Pennsylvania State University Agricultural Experimental Station at Rock Springs, Pennsylvania.

	Mean (S.E.)	Range
Flower Production	370.6 (± 17.5)	12–1569
Fruit Production	26.4 (± 1.2)	2–89
Seed Production	82.6 (± 3.8)	4–312

We also examined the phenological pattern of *P. coccineus*. We found that *P. coccineus* needed at least 5–6 weeks after emergence for plants to start producing flowers. Furthermore, the time between plant emergence and the production of the first flower was negatively correlated with plant size ($p < 0.002$). The flowering period lasted 10–12 weeks, and followed a sigmoidal pattern that could be divided into three different phases (Figure 1). During the first phase, flower production is very low. On average, only about 10% of the total number of flowers are produced during this phase of the flowering period, which lasts about 5 weeks. During the second phase, lasting 4 to 5 weeks, there is a significant increase in the rate of flower production. Finally, during the third phase, flower production slows down again.

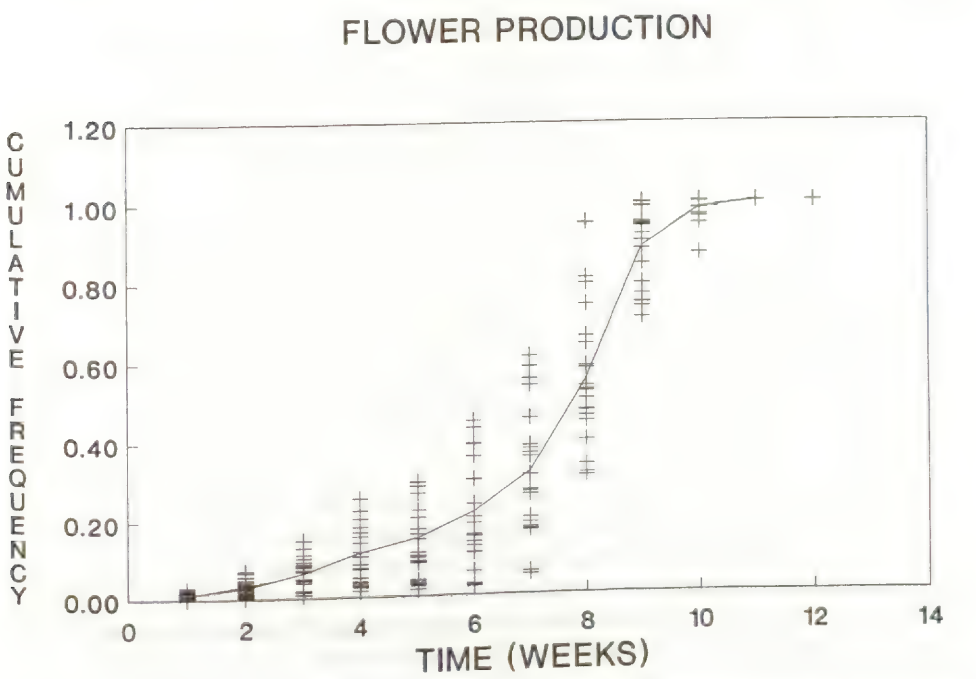


Figure 1. The cumulative frequency of flower production on 20 plants of *Phaseolus coccineus* that were growing in an experimental garden during the summer of 1989.

Others have examined flower production in *Phaseolus vulgaris* (Subhadrabandhu et al., 1978; Pechan and Webster, 1986b) and found that anthesis of the first flower occurs about six weeks after planting. Cumulative leaf area and growth rate increased during the first six

weeks of vegetative growth as a result of continuing leaf initiation (Geiger et al., 1989). For determinate varieties of *P. vulgaris*, flower production lasts for 10–20 days (Pechan and Webster, 1986b; Geiger et al., 1989). Subhadrabandhu et al. (1978) found that in field-grown plants the flowering period extended over a longer time period than in plants grown in a greenhouse. During this period there is an increase in the proportion of newly assimilated carbon that is partitioned to reproduction. The diversion of carbon to the developing young fruits is thought to be a dominant factor in the cessation of flowering and in the increased abortion of flowers observed late in the flowering period (Geiger et al., 1989).

In our experiment, carbon allocation to reproduction increased during the first 8 weeks of the flowering period, as indicated by the number of flowers produced and the presence of developing fruits. In addition, during the second phase of the flowering period, *P. coccineus* also initiates the formation of a tuberous root, where newly assimilated resources are stored. As described in *P. vulgaris*, the developing fruits result in the cessation of flowering. These findings strongly suggest that the phenological pattern of this species is determined by a tradeoff in the allocation of resources between growth and reproduction. In particular, this balance in allocation may be controlling the time of initiation and duration of the flowering period, as well as the number of flowers, fruits, and seeds produced. This finding is not unique to this species. For example, in *Brassica napus*, Tayo and Morgan (1979) reported a reduction of flower and fruit production in experimental plants where the supply of carbon assimilates was reduced by shading or leaf removal (Lovett Doust and Eaton, 1982; see Stephenson, 1981, for review).

EXPERIMENT 2: Determinants of fruit set: availability of pollen.

In order to determine if fruit production was limited by the availability of pollen, we examined pollen deposition on stigmas of *P. coccineus* in our experimental garden at Rock Springs. We collected the stigmatic end of the style of one-day-old flowers, leaving the rest of the flower intact and attached to the plant. The styles were fixed in alcohol (70% v/v). Each experimental flower was marked, censused periodically for fruit development/abortion, and the resulting fruits were harvested at maturity. We examined pollen deposition during phase 1 and phase 2 of the flowering period. Later, the stigmas were softened and cleared in a 8.0N NaOH solution for 24 hours at room temperature. The styles were then rinsed with water, stained overnight in 1.0% aniline blue in a 0.1M K_3PO_4 solution, squashed under a cover slip, and the pollen tubes observed by fluorescent microscopy (Martin, 1959). We counted the number of pollen grains that had germinated on the stigma and produced tubes growing into the style.

We examined 210 styles from flowers collected during phase 1 and 158 styles from phase 2; we found pollen tubes in the styles of most flowers (180 and 137 flowers, respectively; Table 2a). However, only 29 of these flowers resulted in fruits (Table 2b). All of the flowers that resulted in fruits had more than 20 pollen tubes. Our results indicate that nearly 59% of the flowers had more than 20 pollen tubes (i.e., enough pollen deposited onto their stigmas for fruit production; Table 2a). Despite having sufficient pollen, most of these flowers did not set fruit. The proportion of flowers that did not set fruit was larger than the proportion of flowers that received insufficient or no pollen. These findings indicate that pollen deposition did not limit fruit production in our experimental garden.

Others have also determined whether fruit production is limited by the availability of pollen (Bradbury, 1929; Pike, 1982; McDade and Davidar, 1984; Delph, 1986; Snow, 1986; Nybom, 1987; Calvo, 1990; McCarthy and Quinn, 1990; Lyon and Antonovics, 1991; Niesenbaum, 1992; Spira et al., 1992; Stephenson, 1981, 1992). For example, Pechan and Webster (1986) found that in *Phaseolus vulgaris* sufficient pollen grains germinated on

Table 2. a. Number of pollen tubes in the stigma/upper style of flowers produced during the early (phase I) and middle (phase II) phases of the flowering period. b. The proportion of those flowers that produced mature fruit.

a.

Phase	Number of Flowers Examined	Number of Pollen Tubes in Stigma and Upper Style: None	1–20	>20
I	210	30 (14.29%)	55 (26.19%)	125 (59.52%)
II	158	21 (13.29%)	45 (28.40%)	92 (58.23%)

b.

Phase	Number of Flowers Examined	Number of Flowers: No Fruit	Fruit
I	210	198	16 (7.62%)
II	158	145	13 (7.59%)

stigmas to assure fertilization of all ovules. Lee and Bazzaz (1986), working with *Cassia fasciculata*, found that hand-pollination of flowers in the field did not increase the number of mature fruits, suggesting that fruit production is not pollen-limited. Similar results have been reported for other species (Stephenson, 1980; Guth and Weller, 1986). However, other studies have found that fruit production is pollen-limited (Bierzychudek, 1981; Calvo, 1990; Robertson and Wyatt, 1991; Bertness and Shumway, 1992; Karoly, 1992; Schmidt and Antlfinger, 1992).

EXPERIMENT 3: Determinants of fruit set: type of pollen.

In order to examine the effect of the type of pollen deposited onto the stigma on the probability of fruit set, six plants (genets) were grown in a greenhouse in the spring of 1989. Once the plants reached a height of at least 0.70 m, we propagated them vegetatively by cutting pieces from their stems and inducing the formation of adventitious roots with a 300 ppm solution of indolebutyric acid. The cuttings were then transplanted into plastic pots containing perlite. Two weeks later, cuttings (clones) of the five genets that had produced the most cuttings with adventitious roots were transplanted to an experimental garden at Rock Springs. The plants were grown 1 m apart and interspersed in a population of 68 unrelated plants that served as sources of pollen.

In late July, we performed a series of control pollinations on the five genets described above. Each day we selected flowers that would open the following day and covered them with a cheesecloth bag. On the day of anthesis, we conducted three types of hand-pollinations: (1) all the flowers in the inflorescence received pollen from the same plant (i.e., self-pollinations), (2) all the flowers on the inflorescence received pollen from one of five unrelated pollen donors (every plant received pollen from all five donors), and (3) all flowers on the inflorescence received a mixture of pollen from three unrelated pollen donors. To conduct the hand-pollinations the left wing petal was pressed downward, causing the pollen-free stigma to emerge from the keel. Because the anthers of *P. coccineus* dehisce the evening before anthesis and the pollen is released onto a brush of hairs on the style, pollen could be deposited by rubbing these pollen-loaded style brushes against the receptive stigma. Pollen from three different donors was deposited by rubbing simultaneously three of these pollen-loaded styles against the stigma. All pollinations were conducted between 8:00 and

10:00 AM over a seven-day period. Twenty-four hours after pollination the stigmatic end of the style was collected and fixed in 70% alcohol (v/v), cleared, stained with aniline blue, and examined with a fluorescence microscope as described above.

The results of this experiment are shown in Table 3. We conducted 250 pollinations of each type. Most of the stigmas examined had more than 20 pollen tubes growing through the style. Our data showed that self-pollinated flowers had the lowest probability of fruit production, with only two fruits produced (0.8%). Both types of cross pollinations resulted in higher fruit set (7.2 and 10.4%). Contingency table analysis revealed that the probability of fruit production was independent of the number of non-self donors (one vs. three) involved in the cross. Furthermore, manual cross-pollination of flowers did not increase the proportion of fruits matured with respect to that found in naturally pollinated flowers. Thus, *P. coccineus* is highly self-sterile but in our population the ability to garner cross pollen did not limit fruit maturation.

Table 3. Fruit and seed production resulting from self-, single donor-, and multiple donor-pollinations.

Type of Cross	Number of Pollinations	Number of Fruits [a,b]	% Fruit Set	Mean Seed per Fruit	Number of Pollen Tubes in the Style
Self	250	2	0.8	1.50	—
Single Donor	250	18	7.2	3.11	30.6
Multiple Donors	250	26	10.4	3.15	29.3

[a] Number of fruits vs. pollinated flowers without fruits on all three types of crosses. chi-square = 19.79; df = 2; $P < 0.0001$.

[b] Number of fruits vs. pollinated flowers without fruits on single and multiple donor crosses only. chi-square = 1.59; df = 1; $P > 0.10$.

Other authors have suggested that the source of pollen could influence the probability of fruit maturation (Janzen, 1977; Charnov, 1979; Stephenson and Bertin, 1983; Bookman, 1984; Lee, 1988). The effect of pollen source on fruit maturation has been well documented for self-incompatible species, where fertile hermaphroditic plants fail to produce fruits upon self-pollination (Stephenson, 1981; Barrett, 1988; Lee, 1988; Barrett et al., 1989). The mechanisms of self-incompatibility may operate before fertilization (prezygotic incompatibility) or after fertilization (postzygotic incompatibility) (Lee, 1988). But, self-incompatibilities are sometimes only partial, with at least a small amount of fruit production occurring after selfing. However, fruits from self-pollinated flowers tend to have fewer seeds and are more likely to abort than fruits from cross-pollinated flowers (Stephenson, 1981; Bookman, 1984; Stephenson and Bertin, 1983; Lee, 1988; Vander Kloet, 1991; Becerra and Lloyd, 1992).

Differential maturation may also occur among fruits initiated with pollen from different donors (Bertin, 1982, 1988; Bookman, 1984; Marshall and Ellstrand, 1985, 1988; Lee, 1988). For example, Bookman (1984) showed that in *Asclepias speciosa*, certain pollen donors always produced more fruits than others across a number of maternal recipients. This indicated that a genetic factor (paternal effect) can affect the number of fruits sired by donors. Bertin (1982) showed that in *Campsis radicans*, pollen recipients differentially mature fruits

sired by certain donors, and that donors that are more successful in siring fruits on one recipient plant may not be as successful on others. Thus, fruit production may also be a consequence of genotype interactions between paternal and maternal parents. Similar findings have been reported for *Raphanus sativus* (Marshall and Ellstrand, 1985, 1988).

Bertin (1986) conducted a study to determine the influence of single- and multiple-donor pollen loads on fruit production in *Campsis radicans*. He found that the number of pollen donors had no effect on the proportion of fruit produced. However, he noted that in single-donor pollinations, the identity of the donor did have a strong effect on fruit production. Marshall and Ellstrand (1985, 1988) reported similar results for *Raphanus sativus*, where multiple donors resulted in greater total seed mass per fruit, but did not increase the proportion of fruits produced.

Other authors noted the relationship between the number of pollen grains that are deposited onto the stigma and the likelihood of fruit maturation (McDade and Davidar, 1984; Winsor et al., 1987, Stephenson et al., 1988). In general, flowers that receive high pollen loads (high number of pollen grains in relation to the number of ovules in the ovary) are more likely to mature fruits than flowers that receive low pollen loads. Furthermore, high pollen loads typically result in fruits with more seeds. Stephenson et al. (1988) examined the effects of seed number on the likelihood of fruit maturation by manipulating the number of pollen grains deposited onto stigmas of zucchini squash. They found that the number of seeds in the prior developing fruit has a significant effect on the probability of flower production and fruit abortion. They also noted that when the number of seeds varied among the fruits on a vine the fruits with the fewest seeds (smallest pollen loads) were most likely to abort.

EXPERIMENT 4: Pattern of flower, fruit, and seed production within inflorescences.

In 1989, we studied the patterns of flower, fruit, and seed production within inflorescences of *P. coccineus*. First, we marked 1000 inflorescences on plants growing in the experimental garden described in experiment 1. Flowers on these inflorescences were permitted to be open-pollinated and the resulting fruits were harvested at maturity. Within each inflorescence, we recorded the number of fruits produced and the node at which each fruit was produced. Second, we monitored daily flower production on 70 of the 1000 inflorescences. We marked every flower and recorded the node within the inflorescence where each fruit was produced.

Our data revealed that most inflorescences (63.7%) failed to produce fruits (Table 4). In addition, we found that of the 363 inflorescences that produced fruits, 251 produced only 1 or 2. Overall, each of the 1000 inflorescences produced, on average, 1.45 fruits.

Table 4. The number of mature fruits per inflorescence from a sample of 1000 inflorescences.

	Number of Fruits per Inflorescence								
	0	1	2	3	4	5	6	7	8
Frequency	637	130	121	64	34	8	4	1	1

Of the 70 inflorescences in which we monitored daily flower production, only 30 produced at least one fruit. The remainder either failed to produce a fruit (30) or were damaged or lost during the study period (10). Daily flower production remained fairly constant on those inflorescences that failed to produce fruits (Table 5a). On average, these

inflorescences produced 0.8 flowers a day. In contrast, daily flower production showed a different pattern in those inflorescences with developing fruits (Table 5b). There was a significant reduction in daily flower production beginning eight days after the initiation of the first fruit on the inflorescence. On average, daily flower production was only 0.2, i.e., one flower every 4 or 5 days. We also found that the flowers produced during this period (days 8 through 15) had a lower probability of fruit set than flowers produced before or after that period (data not shown). Our results revealed that the presence of developing fruits significantly reduced daily flower production within the inflorescence. In addition, flowers produced on inflorescences with 8- to 15-day-old developing fruits are less likely to set fruits than flowers produced at other times or on inflorescences without fruits.

Table 5. a. Mean daily flower production in 30 inflorescences that failed to produce fruit. b. Mean daily flower production after the initiation of the first fruit in 17 inflorescences that initiated fruits. Experiment conducted at Rock Springs, Pennsylvania, 1989.

a.

	Inflorescences Without Fruit Days After Anthesis of First Flower													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mean Flower Production	0.8	0.8	0.7	0.9	0.8	0.8	0.8	0.7	0.8	0.7	0.9	0.8	0.9	0.6

b.

	Inflorescences With Fruit Days After Initiation of First Fruit													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mean Flower Production	0.7	0.6	0.8	0.8	0.9	0.7	0.6	0.3	0.2	0.25	0.2	0.25	0.25	0.2

In the fall of 1992, we conducted a similar experiment in Costa Rica. This time we grew 15 plants of a local land-race of *P. coccineus* in an experimental garden at Moravia, Costa Rica. We obtained similar results to those described from our experimental population at Rock Springs. Our data reveal that flower production remained constant in inflorescences that did not produce fruits (Table 6a); however, flower production significantly declined in those inflorescences with developing fruits (Table 6b). Furthermore, the period when flower and fruit production declined coincides with the period when the fruit increases most in length (Figure 2). These findings suggest that during the period of high demand for nutrients and/or carbohydrates by the developing fruits, the production of flowers, fruits, and seeds is inhibited. Apparently, it is limited by the availability of resources.

Several studies have shown that the production of flowers, fruits, and seeds can be affected by the presence of developing fruits (Stephenson, 1981; Lee, 1988 for reviews). For example, in *Phaseolus vulgaris*, where anthesis occurs acropetally within the inflorescence, the probability of flower bud and fruit abortion varies in relation to its position. In general, basal buds are first to flower and are less likely to abort than distal buds (Sage and Webster, 1987). Furthermore, pods retained to maturity typically originate from flowers that open within five days of the anthesis of the inflorescence and are located at the basal positions (Pechan and Webster, 1986b). Similar patterns have also been reported for other perfect-flowered plants such as soybeans (Heindly and Brun, 1984), wild radish (Stanton et al., 1987), and *Campanula americana* (Richardson and Stephenson, 1991).

Table 6. a. Mean daily flower production in 54 inflorescences that failed to produce fruit. b. Mean daily flower production after the initiation of the first fruit in 34 inflorescences that initiated fruits. Experiment conducted at Moravia, Costa Rica, 1992.

a.	Inflorescences Without Fruit												
	Days After Anthesis of First Flower												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean Flower Production	1.8	1.2	1.5	1.7	1.9	2.3	1.9	1.5	1.8	1.7	1.6	1.3	1.5

b.	Inflorescences With Fruit												
	Days After Initiation of First Fruit												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean Flower Production	1.3	1.8	1.5	1.6	1.7	1.5	0.5	0.5	0.2	0.3	0.1	0.3	0.1

Other studies have shown that the removal of basal buds and fruits leads to more flowers (Tayo and Morgan, 1979; Lloyd, 1980; Lovett Doust and Eaton, 1982; Stephenson et al., 1988). In addition, in other species the removal of rapidly growing fruits resulted in growth and maturation of fruits that normally would not have matured (Lee and Bazzaz, 1982, 1982b; Egli et al., 1985; Stephenson et al., 1988). The reasons for the abortion of

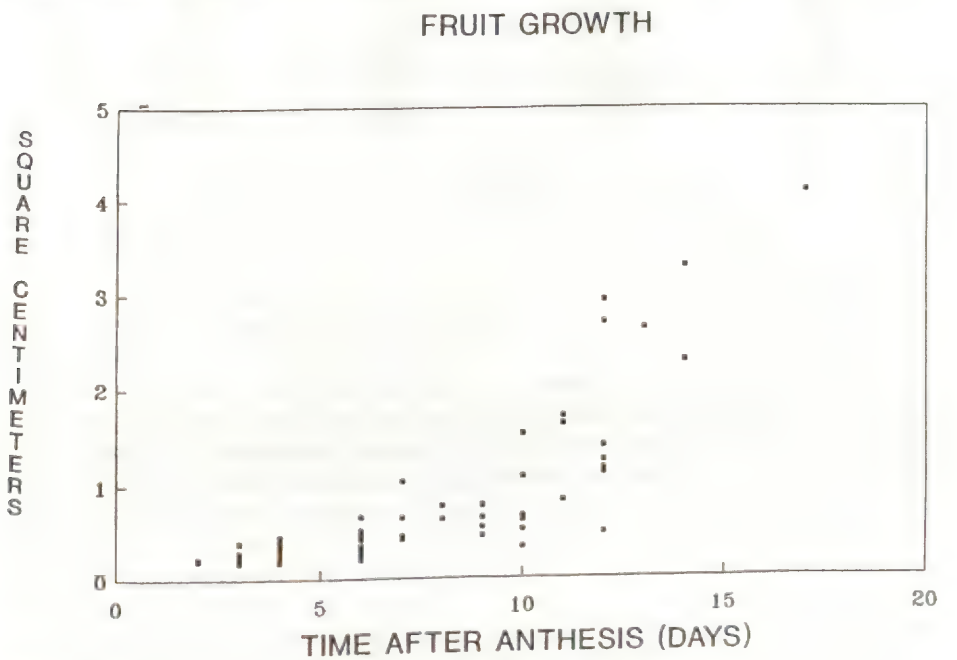


Figure 2. The changes in pod size (width × length) with age. Time 0 is the day of anthesis.

flower buds, flowers, and fruits are not known, but the high demand for nutrients by the developing fruit is one possible explanation (Adedipe and Ormrod, 1975; Sachs and Hackett, 1981). Tamas et al. (1979) reported that, in *Phaseolus vulgaris*, the removal of the older fruits at the base of the racemes reduced fruit abortion rate of the younger fruits and their abscisic acid concentration. These findings suggest that the production of plant hormones regulates source/sink interaction and effectively diverts resources away from younger fruits. More recent studies tend to support this notion (Bangerth, 1989; Lepage-Degivry et al., 1989). However, our understanding of the physiology of fruit growth/abortion is still incomplete and the results are occasionally contradictory (Adedipe et al., 1976).

We also examined the effect of the position of the fruit within the inflorescence on seed number. We found that, on average, the fruits of *P. coccineus* produce approximately three seeds per fruit, regardless of their position within the inflorescences or the number of fruits on the inflorescence (Figure 3). We found only a slight decrease in the number of seeds per fruit on the fourth fruit of inflorescences with 4 or more fruits.

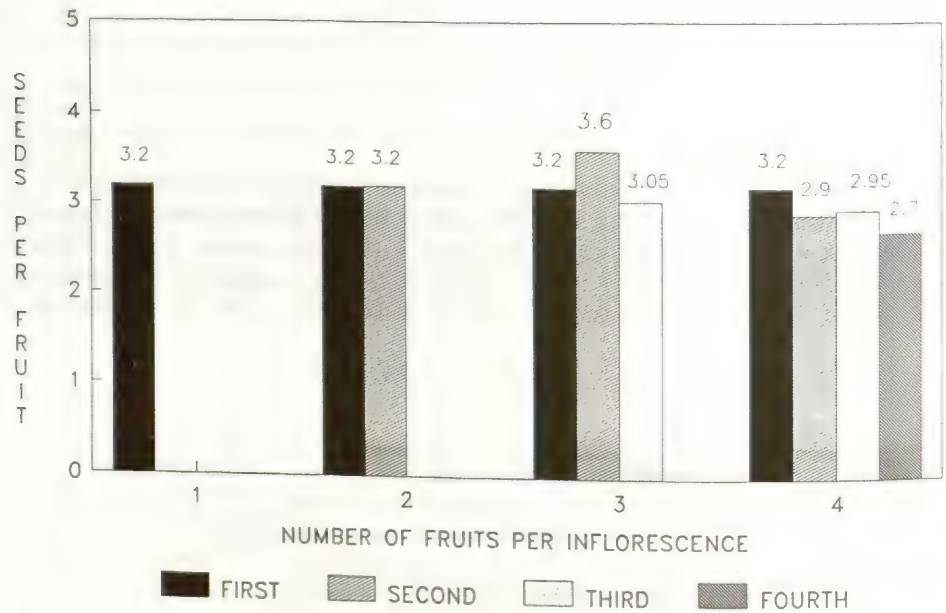


Figure 3. The number of seeds per fruit on inflorescences of *Phaseolus coccineus* producing one, two, three, or four or more fruits. Bars indicate mean number of seeds in the first, second, third, and fourth fruit.

Many studies have also reported a decrease in seed number and mean seed weight per fruit in fruits produced later in the growing season (Stephenson, 1981; Lee, 1988). For example, in *Lobelia cardinalis*, which produces terminal inflorescences that develop acropetally, seed number and mean seed weight varied systematically with floral position on the inflorescence. The flowers in the top one-third of the inflorescence produced significantly fewer and smaller seeds than the flowers on the bottom third of the inflorescence (Devlin, 1989). Often such changes in seed number and seed weight per fruit are reversible if fruit set is prevented on the earlier flowers, indicating that prior fruit and seed production has a negative effect on the later fruits, probably by depleting the resources needed for fruit and seed maturation (van Steveninck, 1957; Galen et al., 1985; Marshall et al., 1985; Stanton et al., 1987). The reduction in seed number per fruit could potentially be due to either a decrease

in number of ovules per flowers or an increase in seed abortion (Pechan and Webster, 1986, 1986b; Stanton et al., 1987; Vasek et al., 1987; Thomson, 1989; Sage and Webster, 1987; Rocha and Stephenson, 1990, 1991).

EXPERIMENT 5: Patterns of seed production within fruits.

In order to determine the patterns of seed production within fruits, we conducted a field experiment in the summers of 1987, 1988, and 1989. We vegetatively propagated three (1987) or six (1988, 1989) individuals (genets) as described for experiment 3. We transplanted six individual cuttings (ramets) from each genet to our experimental garden at Rock Springs. We allowed flowers to be naturally pollinated.

Every fruit produced by each plant was harvested, and the position of each seed within the ovary was scored. Because of the linear arrangement of the ovules within the fruit, it was possible to number the ovules from the most distal (stylar end) to the most proximal (peduncular end). Even when ovules did not produce mature seeds, it was possible to identify their position within the fruit.

In addition, in 1989 we also recorded the number of unexpanded ovules (translucent yellow in color) and the number of expanded ovules that failed to produce seeds. Detailed studies have shown that unexpanded ovules were unfertilized, whereas expanded ovules were aborted seeds that failed to produce mature seeds (Stoddard, 1986; Lee and Bazzaz, 1988; Rocha and Stephenson, 1991b). Consequently, we could calculate the number of ovules fertilized in each ovule position.

Fruits examined in 1987, 1988, and 1989 contained approximately three seeds per fruit (Table 7). These findings indicate that about 50% of the available ovules failed to produce seeds. Furthermore, we found that there is a significant effect of ovule position within the fruit on the probability of seed maturation. In general, the three stylar ovule positions had a higher probability of seed maturation than the three basal ovule positions (Figure 4; Rocha and Stephenson, 1990, 1991). We also found that 98% of the 4512 ovules contained in the 752 fruits examined in 1989 were fertilized (Rocha and Stephenson, 1991b). These findings indicate that seed number per fruit is not limited by the rate of fertilization; instead, more ovules are fertilized than seeds matured.

Table 7. Mean number of seeds per fruit from plants grown in three consecutive years in an experimental garden at The Pennsylvania State University Agricultural Experimental Station at Rock Springs, Pennsylvania.

Year	Mean Seed Number/Fruit	Number of Fruits Examined
1987	3.18 ± 0.41	102
1988	3.11 ± 0.26	309
1989	2.93 ± 0.18	884

Many plant species produce more ovules than mature seeds (Stephenson, 1981; Wiens, 1984; Lee, 1988). Sometimes ovules fail to produce seeds because pollination /fertilization has not occurred or as a result of external factors such as seed predation (Stephenson, 1981; Delph, 1986; Lee and Bazzaz, 1986; Nakamura, 1986; Lee, 1988), but a few recent studies have shown that a large fraction of the fertilized ovules often fail to mature seeds in many species (Nakamura, 1983; Casper, 1984; Guth and Weller, 1986). For example, all four ovules of *Cryptantha flava* are typically fertilized; however, most fruits contain only one seed (Casper, 1984). Pechan and Webster (1986) reported that all ovules examined in

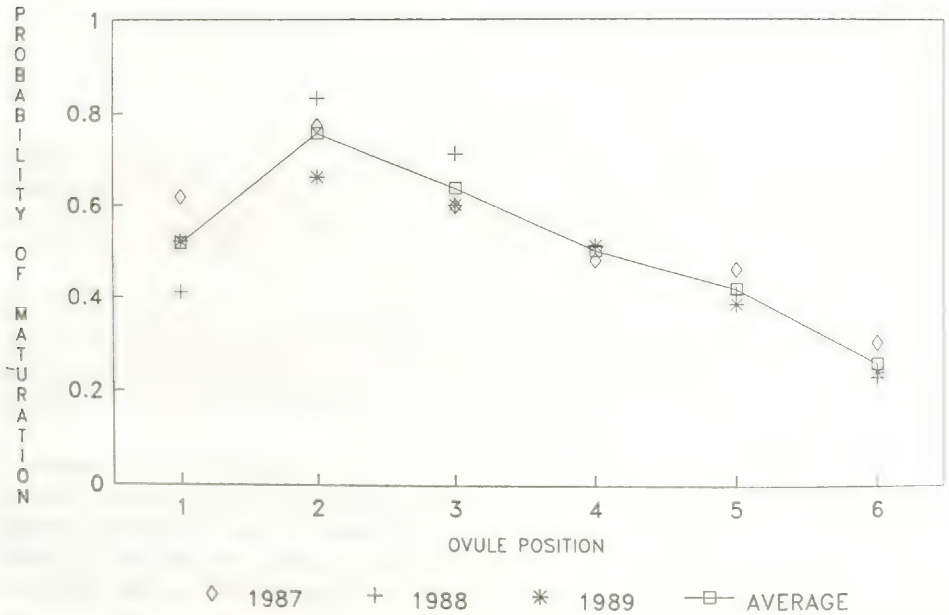


Figure 4. The probability of seed maturation for ovules in the different positions within the fruit (1 is the stylar end, 6 is the peduncular end) in three different years.

Phaseolus vulgaris were fertilized, but they found a discrepancy between the number of ovules fertilized and the final seed number per pod. In addition, Nakamura (1983, 1986) reported that the basal ovules within the ovary of *P. vulgaris* are less likely to mature seeds. However, these normally aborted ovules develop viable embryos when cultured *in vitro*. Lee and Bazzaz (1986) reported that about 95% of the ovules of *Cassia fasciculata* were fertilized, but only about 53% of the ovules in mature fruits matured seeds. They also pointed out that the ovules near the base of the fruit were less likely to mature seeds than ovules at the stylar end. These studies concluded that such difference between fertilized ovules and seed matured are primarily due to the abortion of ovules (Bawa and Webb, 1984). In addition, Nakamura (1983, 1986) and Lee and Bazzaz (1986) suggested that resources limit seed maturation per fruit and promote seed abortion (Stephenson, 1981, 1992; Nakamura and Stanton, 1987; Lee, 1988; Stephenson et al., 1988).

Several studies have indicated that seed abortion is nonrandom with respect to the position of the ovule within the ovary, especially in species with linear arrangement of the ovules such as legumes (Halsten, 1917; Horovitz et al., 1976; Wyatt, 1981; Bawa and Webb, 1984; Harper and Wallace, 1987; Hossaert and Valéro, 1988; Nakamura, 1988). For example, in *Medicago sativa* the even-numbered ovule positions are the most likely to mature (Horovitz et al., 1976). In *Phaseolus vulgaris*, the ovules at the stylar end of the ovary are most likely to produce mature seeds when selfed or when crossed with another plant of the same cultivar, but the position effect disappears when crossed to another cultivar (Nakamura, 1988). Harper and Wallace (1987) found that in *Epilobium montanum* the basal ovule positions were less likely to produce seeds following self-pollination, but the position effect was even stronger following cross-pollination. These last studies indicate a genetic component to the position effect. In addition, Gabelman and Williams (1962), Kambal (1969), and Rocha and Stephenson (1991b) proposed that in legumes, the time and order of fertilization of the ovules within the fruit is critical for the survival of the embryo.

SUMMARY AND CONCLUSION

We examined several factors that affect the production of flowers, fruits, and seeds in *P. coccineus*. We found that fruit and seed production is resource-limited and that more flowers are produced and pollinated, and more ovules are fertilized than mature fruits and seeds. Furthermore, the manual addition of pollen to flowers in the field did not increase the number of fruits matured, suggesting that fruit production was not pollen-limited. Instead, the presence of developing fruits on the inflorescence typically reduced flower production and also reduced the probability that flowers would set fruit.

There is ample evidence to suggest that resource limitation leads to fruit and seed abortion (reviews by Stephenson, 1981, 1992; Lee, 1988). In some studies of yield components, for example, a reduction in one component of yield (such as fruit number) results in an increase in another component of yield (such as seed number per fruit; Adams, 1967; Lovett Doust and Eaton, 1982; Vasek et al., 1987). Other studies have shown that the probability of fruit maturation and seed number per fruit decreases with the presence of older fruits developing on the inflorescence (e.g., Adams, 1967; Sheldrake and Saxena, 1979; Harper and Wallace, 1987; Nakamura, 1988; Stephenson et al., 1988), or that the reduction in the number of developing fruits can increase flower production and reduce fruit and seed abortion (Tayo and Morgan, 1979; Lee and Bazzaz, 1982, 1982b, 1988). Presumably, older fruits are better competitors (sinks) for maternal resources by virtue of their age or position within the inflorescence, and their removal avails resources for subsequently formed flowers and fruits (Sheldrake and Saxena, 1979; Pechan and Webster, 1986, 1986b; Sage and Webster, 1987; Stephenson et al., 1988).

The addition of resources, such as water and nutrients, decreases fruit and seed abortion in some species (Gabelman and Williams, 1962; Stephenson, 1981, 1992; Casper, 1984; Nakamura and Stanton, 1987). However, in other species, seed abortion is unaffected by additional resources although other components of yield (e.g., fruit production) may respond (Weller, 1980; Lovett Doust and Eaton, 1982; Pechan and Morgan, 1983; Casper, 1984; Marshall et al., 1985). Experimentally applied stress, such as that caused by drought or defoliation, often increases seed abortion, especially when applied after ovule fertilization /fruit initiation but before seed filling (Janzen, 1976; Lee and Bazzaz, 1986; Marshall et al., 1986; Harper and Wallace, 1987).

In conclusion, in plants in which reproduction is resource-limited, the patterns of flower production and fruit and seed abortion are not random. Even though plants have little or no control over the number or the genotype of the pollen grains deposited onto their stigmas, it is becoming increasingly clear that plants can regulate, at least to some degree, the quality of their progeny (Stephenson, 1981; Marshall and Ellstrand, 1985, 1986, 1988; Bertin, 1988; Stephenson and Winsor, 1986; Winsor et al., 1987; Lee, 1988; Rocha and Stephenson, 1991). Other authors have reviewed the possible role of the overproduction of flowers, fruits, and seeds in plants (Stephenson, 1981; Lee, 1988). Some have argued that the overproduction of fruits and ovules followed by nonrandom abortion of fruits/seeds is a mechanism that increases the average quality of the seed crop (Janzen, 1976; Charnov, 1979; Westoby and Rice, 1982; Bawa and Webb, 1984; Bookman, 1984; Stephenson and Winsor, 1986; Winsor et al., 1987). This hypothesis does not, however, assume that plants actually choose which fruits and seeds to mature and which to abort. The fruits and seeds are nearby resource "sinks," competing with one another as well as with vegetative "sinks." The success of each fruit and each seed within a fruit is a function of their ability to compete for maternal resources (Lee, 1988; Stephenson, 1992).

LITERATURE CITED

Adams, M. W. 1967. Basis of yield components in crop plants with special reference to the field bean, *Phaseolus vulgaris*. Crop Sci. 7: 505–510.

Adedipe, N. O., R. A. Fletcher, and D. P. Ormrod. 1976. Distribution of ^{14}C assimilates in the cowpea (*Vigna ungulata* L.) in relation to fruit abscission and treatment with benzyladenine. Ann. Bot. 40: 731–737.

_____ and D. P. Ormrod. 1975. Absorption of foliar-applied ^{32}P by successive leaves, and distribution patterns in early fruiting and abscission in the cowpea (*Vigna ungulata* L.) Ann. Bot. 39: 639–646.

Bangerth, F. 1989. Dominance among fruits/sink and search for correlative signal. Physiol. Plantarum 76: 608–614.

Barrett, S. C. H. 1988. The evolution, maintenance, and loss of self-incompatibility systems. Pp. 98–124 *in* J. Lovett Doust and L. Lovett Doust (eds.), Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, Oxford.

Bawa, K. S. and C. J. Webb. 1984. Flower, fruit, and seed abortion in tropical trees: implications for the evolution of paternal and maternal reproductive patterns. Amer. J. Bot. 71: 736–751.

_____, S. G. Hedge, K. N. Ganeshaiah, and R. U. Shaanker. 1989. Embryo and seed abortion in plants. Nature 342: 625.

Becerra, J. X. and D. G. Lloyd. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormum tenax* (Agavaceae): a second action of self-incompatibility at the whole flower level. Evolution 46: 458–469.

Bertin, R. I. 1982. Paternity and fruit production in trumpet creeper (*Campsis radicans*). Amer. Nat. 119: 694–709.

_____. 1986. Consequences of mixed pollinations in *Campsis radicans*. Oecologia 70: 15.

_____. 1988. Paternity in plants. Pp. 30–50 *in* J. Lovett Doust and L. Lovett Doust (eds.), Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, Oxford.

_____. 1990. Effects of pollination intensity in *Campsis radicans*. Amer. J. Bot. 77: 178–187.

Bertness, M. D. and S. W. Shumway. 1992. Consumer driven pollen limitation of seed production in marsh grasses. Amer. J. Bot. 79: 288–293.

Bierzuchudek, P. 1981. Pollinator limitation of plant reproductive effort. Amer. Nat. 117: 838–840.

Bookman, S. S. 1984. Evidence for selective fruit production in *Asclepias*. Evolution 38: 72–86.

Bradbury, D. 1929. A comparative study of the developing and aborting fruits of *Prunus cerasus*. Amer. J. Bot. 16: 525–542.

Calvo, R. 1990. Four year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in south Florida. Amer. J. Bot. 77: 736–741.

Casper, B. B. 1984. On the evolution of embryo abortion in the herbaceous perennial *Cryptantha flava*. Evolution 38: 1332–1349.

Charnov, E. L. 1979. Simultaneous hermaphroditism and sexual selection. Proc. Natl. Acad. Sci. USA 76: 2480–2484.

Delph, L. F. 1986. Factors regulating fruit and seed production in the desert annual *Lesquerella gordonii*. Oecologia 69: 471–476.

Devlin, B. 1989. Components of seed and pollen yield of *Lobelia cardinalis*: variation and correlations. Amer. J. Bot. 76: 204–214.

Egli, D. B., R. D. Guffy, L. W. Meckel, and J. E. Leggett. 1985. The effects of source-sink alterations on soybean seed growth. Ann. Bot. 55: 395–402.

Gabelman, W. H. and D. D. F. Williams. 1962. Water relationships affecting pod set of green beans. Pp. 321–325 *in* Proc. Campbell Soup Co. Plant Sci. Symp, Camden, N.J.

Galen, C., R. C. Plowright, and J. D. Thomson. 1985. Floral biology and regulation of seed set and seed size in the lily *Clintonia borealis* (Ait.) Raf. Amer. J. Bot. 72: 1544–1552.

Geiger, D. R., W. J. Shieh, and R. M. Saluke. 1989. Carbon partitioning among leaves, fruits, and seeds during development of *Phaseolus vulgaris* L. Plant Physiol. 91: 291–297.

Goldman, D. A. 1990. Minimal male/female tradeoffs in *Zizania palustris*, a monoecious annual grass. Amer. J. Bot. 78: 189–197.

Guth, C. J. and S. G. Weller. 1986. Pollination, fertilization, and ovule abortion in *Oxalis magnifica*. Amer. J. Bot. 73: 246–253.

Halsted, B. D. 1917. Abortiveness as related to position in the legume. Proc. 38th Ann. Meet. Soc. Prom. Agric. Sci. 68–72.

Harper, J. L. and H. L. Wallace. 1987. Control of fecundity through abortion in *Epilobium montanum* L. Oecologia 74: 31–38.

Heindy, J. L. and W. A. Brun. 1984. Patterns of reproductive abscission, seed yield, and yield component in soybean. Crop Sci. 24: 1544–1552.

Horovitz, A., L. Meiri, and A. Beiles. 1976. Effects of ovule position in fabaceous flowers on seed set and outcrossing rates. Bot. Gaz. 137: 250–254.

Hossaert, M. and M. Valéro. 1988. Effects of ovule position in the pod on patterns of seed formation in two species of *Lathyrus* (Leguminosae). Amer. J. Bot. 75: 1714–1731.

Janzen, D. H. 1976. Effect of defoliation on fruit-bearing branches of the Kentucky coffee tree, *Gymnocladus dioicus* (Leguminosae). Amer. Midl. Nat. 95: 474–478.

_____. 1977. A note on optimal mate selection by plants. Amer. Nat. 111: 365–371.

Kambal, A. E. 1969. Flower drop and fruit set in field beans. J. Agric. Sci. 72: 131–137.

Karoly, K. 1992. Pollination limitation in the facultatively autogamous annual, *Lupinus nanus* (Leguminosae). Amer. J. Bot. 79: 49–56.

Le Page-Degivry, M. Th., Ph. Barthe, I. Prévost, and B. Boulon. 1989. Regulation of abscissic acid translocation during embryo maturation of *Phaseolus vulgaris*. Physiol. Plant. 77: 81–86.

Lee, T. D. 1984. Patterns of fruit maturation: a gametophyte competition hypothesis. Amer. Nat. 123: 427–432.

_____. 1988. Patterns of fruit and seed production. Pp. 179–263 *in* J. Lovett Doust and L. Lovett Doust (eds.), Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, Oxford.

_____ and F. A. Bazzaz. 1982. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. Ecology 63: 1363–1373.

_____ and _____. 1982b. Regulation of the fruit maturation patterns in an annual legume, *Cassia fasciculata*. Ecology 63: 1374–1388.

_____ and _____. 1986. Maternal regulation of fecundity: non-random ovule abortion in *Cassia fasciculata*. Oecologia 68: 459–465.

Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytol. 86: 69–80.

Lovett Doust, J. and G. W. Eaton. 1982. Demographic aspects of flower and fruit production in bean plants, *Phaseolus vulgaris* L. Amer. J. Bot. 69: 1156–1164.

Lyons, E. E. and J. Antonovics. 1991. Breeding system evolution in *Leavenworthia*: breeding system variation and reproductive success in natural populations of *Leavenworthia crassa* (Cruciferae). Amer. J. Bot. 78: 270–287.

Marshall, D. L. and N. C. Ellstrand. 1985. Proximal causes of multiple paternity in wild radish, *Raphanus sativus*. Amer. Nat. 127: 446–461.

_____ and _____. 1986. Sexual selection in *Raphanus sativus*: experimental data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. Amer. Nat. 127: 446–461.

_____ and _____. 1988. Effective mate choice in wild radish: evidence for selective seed abortion and its mechanisms. Amer. Nat. 131: 739–756.

_____, D. A. Levin, and N. L. Fowler. 1985. Plasticity of yield components in response to fruit predation and date of fruit initiation in three species of *Sesbania* (Leguminosae). J. Ecol. 73: 71–80.

_____, _____, and _____. 1986. Plasticity of yield components in response to stress in *Sesbania macrocarpa* and *Sesbania vesicaria* (Leguminosae). Amer. Nat. 127: 508–521.

Martin, F. M. 1959. Staining and observation of pollen tubes by means of florescence. Stain Technol. 34: 436–437.

McCarthy, B. C. and J. A. Quinn. 1990. Reproductive ecology of *Carya* (Juglandaceae): phenology, pollination, and breeding system of two sympatric tree species. Amer. J. Bot. 77: 261–273.

McDade, L. A. and P. Davidar. 1984. Determinants of fruit and seed set in *Pavonia dasypetala* (Malvaceae). Oecologia 64: 61–67.

Nakamura, R. R. 1983. Reproductive capacity and kinship in *Phaseolus vulgaris* L. Ph.D. Thesis. Yale University, New Haven, CT.

_____. 1986. Maternal investment and fruit abortion in *Phaseolus vulgaris*. Amer. J. Bot. 73: 1049–1057.

_____. 1988. Seed abortion and seed weight variation within fruits of *Phaseolus vulgaris*: pollen donor and resource limitation effects. Amer. J. Bot. 75: 1003–1010.

_____ and M. L. Stanton. 1987. Cryptic seed abortion and the estimated ovule fertilization. Canad. J. Bot. 65: 2463–2465.

Niesenbaum, R. A. 1992. Sex ratio, components of reproduction, and pollen deposition in *Lindera benzoin* (Lauraceae). Amer. J. Bot. 79: 495–500.

Nybom, H. 1987. Pollen limited seed set in pseudogamous blackberries (*Rubus* L. subgen. *Rubus*). Oecologia 72: 562–568.

Pechan, P. M. and B. D. Morgan. 1983. The use of radiography in studies of plant morphology *in vivo*. Planta 159: 476–481.

_____ and B. D. Webster. 1986. Seed and pod set of red kidney beans. J. Amer. Soc. Hort. Sci. 111: 87–89.

_____ and _____. 1986b. Flower and pod set of *Phaseolus vulgaris* under controlled environment conditions. HortScience 21: 989–991.

Pike, G. H. 1982. Fruit set in *Lambertia formosa* Sm. (Proteaceae). Austral. J. Bot. 30: 39–45.

Richardson, T. E. and A. G. Stephenson. 1991. Effects of pollen parentage, prior fruit set, and pollen load on fruit and seed production in *Campanula americana* L. Oecologia 87: 80–85.

Robertson, J. L. and R. Wyatt. 1990. Reproductive biology of the yellow-fringed orchid, *Platanthera ciliaris*. Amer. J. Bot. 77: 388–398.

Rocha, O. J. and A. G. Stephenson. 1990. Effects of ovule position on seed production, seed weight, and progeny performance in *Phaseolus coccineus* L. (Leguminosae). Amer. J. Bot. 77: 1320–1329.

_____ and _____. 1991. Effects of nonrandom seed abortion on progeny performance in *Phaseolus coccineus* L. Evolution 45: 1198–1208.

Rocha, O. J. and A. G. Stephenson. 1991. Order of fertilization within the ovary in *Phaseolus coccineus* L. (Leguminosae). Sex. Plant Reprod. 4: 126–131.

Sachs, R. M. and W. P. Hackett. 1981. Source-sink relationships and flowering. Pp. 263–272 *in* W. J. Meudt (ed.), Strategies of Plant Reproduction. Beltsville Symposium in Agricultural Research. Beltsville, Maryland.

Sage, T. L. and B. D. Webster. 1987. Flowering and fruiting patterns in *Phaseolus vulgaris* L. Bot. Gaz. 148: 35–41.

Schmidt, J. M. and A. E. Antlfinger. 1992. The level of agamospermy in a Nebraska population of *Spiranthes cernua* (Orchidaceae). Amer. J. Bot. 79: 501–507.

Sheldrake, A. R. and N. P. Saxena. 1979. Comparison of earlier and later formed pods of chickpeas (*Cicer arietium* L.). Ann. Bot. 43: 467–473.

Snow, A. A. 1986. Pollination dynamics in *Epilobium canum*: consequences for gametophytic selection. Amer. J. Bot. 73: 139–151.

Spira, T. P., A. A. Snow, D. F. Whigham, and J. Leak. 1992. Flower visitation, pollen deposition, and pollen tube competition in *Hibiscus moscheutos* (Malvaceae). Amer. J. Bot. 79: 428–433.

Stanton, M. L, J. K. Bereczky, and H. D. Hasbrouck. 1987. Pollination thoroughness and maternal yield regulation in wild radish, *Raphanus raphanistrum* (Brassicaceae). Oecologia 74: 68–76.

Stephenson, A. G. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). Ecology 61: 57–64.

_____. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Rev. Ecol. Syst. 12: 253–279.

_____. 1984. The regulation of maternal investment in an indeterminate flowering plant (*Lotus corniculatus*). Ecology 65: 113–121.

_____. 1992. The regulation of maternal investment in plants. Pp. 151–171 *in* C. Marshall and J. Grace (eds.), Fruit and Seed Production: Aspects of Development, Environmental Physiology, and Ecology. Cambridge University Press, Cambridge.

_____ and R. I. Bertin. 1983. Male competition, female choice, and sexual selection in plants. Pp. 109–147 *in* L. Real (ed.), Pollination Biology. Academic Press, New York.

_____, B. Devlin, and J. B. Horton. 1988. The effect of seed number and prior fruit dominance on the pattern of fruit production in *Cucurbita pepo* (zucchini squash). Ann. Bot. 62: 653–661.

_____ and J. A. Winsor. 1986. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. Evolution 40: 453–458.

Stoddard, F. L. 1986. Floral viability and pollen tube growth in *Vicia faba* L. J. Plant Physiol. 123: 249–262.

Subhadrabandhu, S., M. W. Adams, and D. A. Reicosky. 1978. Abscission of flowers and fruits in *Phaseolus vulgaris* L. I. Cultivar differences in flowering patterns and abscission. Crop Sci. 18: 893–896.

Sutherland, S. and L. F. Delph. 1984. On the importance of male fitness in plants: patterns of fruit set. Ecology 65: 1093–1104.

Tamas, I. A., D. H. Wallace, P. M. Ludford, and J. L. Ozbun. 1979. Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris* L. Plant Physiol. 64: 620–622.

Tayo, T. O. and D. G. Morgan. 1979. Factors influencing flower and pod development in oil-seed rape (*Brassica napus* L.). J. Agric. Sci. 92: 363–373.

Thomson, J. D. 1989. Deployment of ovules and pollen among flowers within inflorescences. Evol. Trends Plants 31: 65–68.

Vander Kloet, S. P. 1991. The consequences of mixed pollination on seed set in *Vaccinium corymbosum*. Canad. J. Bot. 69: 2448–2454.

Van Steveninck, R. F. M. 1957. Factors affecting the abscission of reproductive organs in yellow lupine (*Lupinus luteus* L.). I. The effect of different patterns of flower removal. J. Exp. Bot. 8: 373–381.

Vasek, C. F., V. Weng, J. R. Beaver, and K. C. Huszar. 1987. Effects of mineral nutrition on components of reproduction in *Clarkia unguiculata*. Aliso 11: 599–618.

Weller, S. G. 1980. Pollen flow and fecundity in populations of *Lithospermum carolinense*. Amer. J. Bot. 67: 1334–1341.

Westoby, M. and B. Rice. 1982. Evolution of the seed plants and inclusive fitness of plant tissue. Evolution 36: 713–724.

Wiens, D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia 64: 47–53.

Winsor, J. A., L. E. Davis, and A. G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. Amer. Nat. 129: 643–656.

Wyatt, R. 1981. Components of reproductive output in five tropical legumes. Bull. Torrey Bot. Club 108: 67–75.

SYSTEMATICS AND REPRODUCTIVE BIOLOGY[1]

Gregory J. Anderson[2]

ABSTRACT. In spite of a century of recognition, and more than a decade of intensive study by systematists and ecologists, the impact and importance of reproductive characters on systematic treatments is still overlooked or undervalued. Reproductive biology/breeding systems impact systematics in several ways including: (1) dictating processes that in turn can affect patterns, (2) facilitating prediction of patterns of gene flow that thus bear on the question of the nature of species, (3) helping predict the nature and range of variation of populations thus dictating sampling methods, (4) assessing the potential for hybridization, (5) illuminating and evaluating diagnostic characters of systematic importance, and (6) assessing anagenesis in phylogenies. Dramatic examples of the latter two categories are given from representative treatments of species and genera of the Solanaceae, Orchidaceae, and Poaceae. Data on reproductive biology are fundamental for systematic and evolutionary studies.

> "Much of the classification of flowering plants is based on reproductive structures" (Jones and Luchsinger, 1986: 232).
>
> "The most important general point is that the biology of the characters...be determined....The biology of these characters and states must be understood for the much more accurate and deep portrayal of relationships" (Stuessy, 1990: 39).

As these quotes from prominent plant systematics books show, taxonomic treatments generally rely on features associated with the reproductive system, and the biology of such characters must be understood. Historically, at least since John Ray in the early 1700s (e.g., see Jones and Luchsinger, 1986), and subsequently with Linnaeus' "Sexual system" in 1753, systematists have utilized flower and fruit parts, characters of the reproductive system, in systematic treatments. Ornduff (1969), and later Wyatt (1988), discussed the importance of reproductive biology in systematics. Thus it seems obvious, even a truism, that reproductive systems are critical to understand, that novelties in reproductive systems often would be manifest in changes of systematic interpretation, and that this association would be widely appreciated. But is this the situation? An analysis of the major texts in plant systematics—Davis and Heywood (1963), replaced now by the comprehensive Stuessy (1990) as the required reading for graduate students in plant systematics, and Jones and Luchsinger (1986), the newest English language text for undergraduate systematics courses—shows that they include recognition of the importance of reproductive biology in systematics. The books include rather extensive discussion of reproductive biology. However, this topic is not a major component of the chapters on "sources of taxonomic evidence." All three of these important books relegate reproductive biology, in spite of their broad acknowledgment of it, largely to contributions to specific, and more often, infraspecific (population), variability.

[1]I thank Andrew Stephenson for the invitation to speak in the symposium, and Kent Holsinger, Tom Mione, Tom Philbrick, and Carl Schlichting for useful discussions of the topics in the paper. Pati Vitt and Roberta Mason were of considerable help in putting the paper together, and Charles Heiser, Kent Holsinger, Tom Philbrick, Mario Sousa-Peña, and Andy Stephenson made useful comments on the manuscript. I thank the Missouri Botanical Garden, the University of Connecticut, and the National Science Foundation for support of the research and/or participation in the symposium.

[2]Ecology and Evolutionary Biology U-43, University of Connecticut, Storrs, Connecticut 06269-3043, U.S.A.

The more or less exclusive association of reproductive biology with population variability misses the point that changes in compatibility, or rate of outcrossing, or pollination, or dispersal syndromes can have effects that pervade subsequent evolution leading to diversification of lineages at all levels. Perhaps the best example of the broad impact of reproductive characters is that the origin and early diversification of the angiosperms is now well accepted as founded largely on the adoption of biotic pollinators. Acknowledgment of the association of angiosperm diversification and pollinators has a long history itself, dating at least from Darwin in 1877 (Takhtajan [1969] cites a letter from Darwin to G. Saporta), through the "new synthesis" (e.g., Stebbins, 1947; Grant, 1949), to the present (e.g., Basinger and Dilcher, 1984; Crepet and Friis, 1987).

Conversely, there are situations where the floral characters we ordinarily rely on for taxonomic classification contribute primarily to understanding reproductive biology. The examples of such characters obscuring systematic interpretation are legion, but seem still, surprisingly, to be largely underappreciated. Thus, given my own particular bias toward reproductive biology in systematics, and the continuing lack of recognition of the broad contribution that reproductive biology can make to systematics, I review the interdependence between these areas.

Reproductive biology and systematics *sensu lato* (with all the fields taxonomy/systematics has spawned over the last half century—such as population biology, reproductive ecology, molecular systematics, and even parts of molecular evolution) are interdependent. I recognize the following six categories illustrating the relationship between them.

1. Process dictates pattern.

> "Of all the additional approaches which are available to taxonomy today, knowledge of the breeding system is one of the most important, for not only does it affect the pattern of group variation but the evolutionary potentialities of the group concerned" (Davis and Heywood, 1963: 361).

> "The method or methods by which a plant reproduces itself are inseparably connected to the patterns of variability found in the species" (Jones and Luchsinger, 1986: 161).

Approaches to systematics are broadly two-fold: systematists study the pattern of relationships and/or the processes of change that give rise to these relationships. Given the great utility and broad application of cladistic approaches for constructing phylogenetic hypotheses, there is currently more interest in systematics in generation of patterns than in understanding processes. Nonetheless, the study of process remains important *per se*, because it helps in the evaluation of characters employed in the analysis of patterns. A number of systematists, including me, consider character analysis at least as important as the subsequent pattern generation. Pattern and process are obviously related, as Davis and Heywood (1963) and Jones and Luchsinger (1986) recognize, and the pattern, in fact, is in part dictated by the process of change. For example, a group in which speciation is primarily dependent on adaptation to new habitats could manifest quite different patterns than a group where intrinsic chromosomal or incompatibility barriers were the prominent isolating barriers. Thus, understanding breeding systems helps to clarify processes and this in turn aids in understanding the foundation of the pattern of relationships.

Both Davis and Heywood (1963) and Jones and Luchsinger (1986) make the point that the breeding system can make pattern recognition itself difficult. Variation among populations of self-pollinators may be more discontinuous than among populations of outcrossers. This can make recognition of species boundaries perhaps too easy (i.e., it leads to

unsupportable splitting) in selfers (see the *Draba* example below), and more difficult in outcrossers.

2. The nature of species

Features of the breeding system allow predictions of the extent and type of gene flow, phenomena that touch on larger questions like the nature of species: are species a set of coadapted gene complexes maintained by their good fit to a particular niche (or development canalization) or coadapted gene complexes held together by gene flow (Ehrlich and Raven, 1969). Obviously, an understanding of reproductive biology and its relationship to gene flow helps to differentiate between such choices. Hamrick (1987) pointed out that "...accurate estimates of gene flow in plant populations require measures of the mating system, the percentage of selfing and outcrossing...."

Clearly, all characters associated with pollen and seed "flow," the movement of genes among populations, are critical here. A particularly good example with systematic ramifications comes from a study of *Scutellaria* (Lamiaceae) by Olmstead (1989). He concluded that the white flower color shared by three species did not represent a synapomorphy, but was derived independently in all three, and also that two of the three white-flowered species showed higher levels of electrophoretic heterzygosity and polymorphism than their progenitors. He speculated that the latter thus might be associated with altered pollinator behavior effected by the flower color change—this having led to a change in pollen flow and thus to a different population structure.

In the Solanaceae, Mione (1992) has identified a similar phenomenon. Rotate corollas were thought to distinguish the genus *Jaltomata*. Mione now recognizes this as incorrect; the genus includes species with rotate corollas and others with tubular ones, each specialized for pollination by bees and birds, respectively. Such syndromes often yield different patterns of pollen flow.

3. Nature and range of variation.

> "The pattern of this variation is largely determined by the reproductive (breeding) system; it is utilized in adaptive evolutionary processes by natural selection" (Davis and Heywood, 1963: 356).
>
> "The recombination of genetic variability is largely determined by the breeding system...." (Davis and Heywood, 1963: 360).

Perhaps of more direct interest to the practicing systematist is that knowledge of the breeding system gives an opportunity for a better understanding of the nature and range of variation within and between taxa. As Davis and Heywood (1963) recognized, variation is largely determined by the reproductive system. And, as discussed above, variation in selfers and outcrossers is partitioned differently. Hamrick and Godt (1990) report that 51% of the total electrophoretic variation is found among populations of selfers, whereas more than 90% is found within populations of outcrossing (wind-pollinated) species. Schoen and Brown (1991) also show that inbreeding plant species show greater overall electrophoretic variation among populations and greater levels of variation in effective population size (N_e). Therefore, sampling procedures by systematists (and conservation biologists) should be different depending on the breeding system.

Two "classic" examples linking variation and systematics follow. Davis and Heywood (1963) illustrate the ramifications of variation in selfers with a classic study by Alexis Jordan from France, who recognized close to 200 localized species in *Draba* (Cruciferae) in what today is treated as the single highly polymorphic species, *D. verna* L. A newer, but perhaps destined to be classic (for the exciting biology revealed), study involved

species of *Callitriche* with "internal geitonogomy" (Philbrick, 1984). These species are monoecious, but fertilization is quite unusual. Pollen tubes often grow from staminate flowers through vegetative tissue, even through internodal stem regions, to pistillate flowers where they enter the ovary through its base. These obligate selfers will likely show different patterns of variation than an obligate outcrosser like tobacco (from which gametophytic self-sterility (S) alleles were originally described by East and Mangelsdorf in 1925).

Amplifying the point that the partitioning of variation depends on the breeding system is a contribution by Les et al. (1991) analyzing variability, population structure, and conservation biology. On the positive side, they suggest that outcrossers can recover from population bottlenecks more quickly then selfers, but, on the other hand, can fail completely if there are too few outcrossing genotypes, especially if outcrossing is promoted by a sporophytic incompatibility system. Baker (1955) made this point, too, in terms of establishment of new small populations (such as those on islands), arguing that self-incompatibility can create a formidable barrier to sexual reproduction in populations founded by one to a few propagules.

Furthermore, in conservation efforts, sampling that indicates low population diversity might dictate that little genetic material needs to be collected to preserve a taxon (as in Brown and Briggs, 1991). However, indices of variation from morphological and isozyme data are not always compatible (e.g., Holsinger, 1991). As a consequence, if the variation as assayed through morphological or electrophoretic studies does not accurately reflect the breeding system (i.e., specifically alleles at the S locus), populations may be conserved that are too small. With such small populations, it may be difficult or impossible for a self-incompatible species to sexually reproduce (as in Nettancourt, 1977).

4. Breeding systems allow prediction of the incidence of hybridization.

Knowledge of the breeding system, including pollination biology, can also put one in a position to predict the incidence of hybridization, and to take this into account in systematic studies. There is broad recognition that self-incompatibility (based on S genes) also raises at least a partial barrier to interspecific hybridization. In *Solanum* (e.g., Pandey, 1962; Whalen and Anderson, 1981) and in many other angiosperms (Levin, 1978; Lewis and Crowe, 1958; Nettancourt, 1977) there are clear examples of barriers to interspecific hybridization associated with self-compatibility (SC) and incompatibility (SI). The barriers are often unidirectional, preventing SC pollen from germinating on SI stigmas (Lewis and Crowe, 1958), but in other cases there are barriers between SI species (Pandey, 1962). Levin (pers. comm.) reports that interspecific hybrids in *Phlox* are much less likely with SI than with SC species.

5. Evaluation of characters of systematic importance.

The knowledge of reproductive biology is important perhaps most obviously for the illumination it gives to characters of systematic utility *per se*. The vast majority of characters systematists consider important are directly or indirectly related to the breeding system *sensu lato*. And of course it is a truism, but nonetheless important to note, that many of the characters systematists utilize are the same ones that pollinators, not only agents of selection but perhaps the ultimate floral systematists, key on to distinguish the individuals or species on which they choose to forage.

In his early work derived from graduate studies on *Leavenworthia*, Lloyd (e.g., 1965) offered a comprehensive list of characters associated with autogamous species compared with their xenogamous progenitors. Lloyd also focused attention on pollen/ovule ratios that have been useful subsequently in understanding changes in breeding systems and presumed energetic efficiency (e.g., Cruden, 1977; Mione and Anderson, 1992; Philbrick and

Anderson, 1987). Ornduff (1969) expanded on Lloyd's initial character change list, and Wyatt (1988) refined it with further examples. This list of more than 20 characters consists almost entirely of floral features associated with sexual reproduction.

Lloyd (1965), Ornduff (1969), and Wyatt (1988) argued that most floral characters are associated with either changes in pollinator syndromes and/or changes in the breeding system. Examples of the former are given, but the emphasis of discussion is on character-state changes associated with switches from outcrossing to selfing. Ornduff (1969) suggests and Wyatt (1988) shows that among species with reproductive variation, taxonomic treatments emphasizing floral ("reproductive") vs. vegetative (or "non-reproductive") characters could be quite different. With such obvious, clear, and pervasive connections between reproductive biology in all of its manifestations and the associated effects on the characters—primarily flowers and fruits—that we depend on to construct our taxonomic schemes, it seems incumbent on us to understand and consider the reproductive biology of our study organisms in systematic studies.

In my own work in collaboration with R. K. Jansen on *Solanum*, we found that the chloroplast DNA-based cladogram (Anderson and Jansen, in prep.) provided very strong support for the independent origin of two autogamous species that share a range of features—e.g., annual habit, herbaceousness, styles at anther level, and reduced pollen/ovule ratios. It is clear that what might have been viewed as synapomorphic characters instead represent independently derived autoapomorphies as a result of convergence associated with autogamy.

In wild and domesticated species of peanuts (*Arachis* spp., Fabaceae), Lu et al. (1990) found that stigma type did not correlate with taxonomy, genomic groups, or ploidy. Instead, small stigmas (with space for only 1–2 pollen grains) marked perennial species, and larger stigmas (with space for about 15 pollen grains) marked annual species. It also appears from their data that the species with small stigmas all require an insect pollinator (and *may* be self-incompatible), whereas the species with larger stigmas are autogamous. That is, stigma size marks the breeding system. Further, stigma size in this group, and in general I suspect, has important ramifications for population biology. The species with vector-requiring small stigmas also have hairs "guarding" the stigmas, which means that it is difficult to deposit pollen on them, and that the stigmas hold very few grains and can be easily "clogged" by illegitimate pollen. The larger stigmas can, on the other hand, accommodate up to 15 grains, thereby not only allowing easier pollination, but also pollen competition, and a greater range of genetic variability in selection of male parents. Thus, these reproductive characters have ramifications for population biology as well as for systematics.

The larger, better defined, stigmatic surface area of the autogamous *Arachis* species runs contrary to the general pattern in Ornduff's (1969) table comparing the floral traits of autogamous and xenogamous species. Another exception to a general pattern was the discovery by Olmstead of a reversal of the usual trend wherein selfers are derived from outcrossers (see Wyatt, 1988). Olmstead (1989) found that three species in the *Scutellaria angustifolium* complex with a predominantly outcrossing system (and larger corollas) were derived (independently) from primarily selfing species (with smaller corollas). Thus, an apparently useful classification tool—corolla size—is more important in this study as a marker of breeding system evolution than as a marker for the lineage of three species.

In 1947 when Charles Rick was beginning his career-long study of tomatoes, he discovered a gene that caused reduced pubescence on anthers. Plants with this gene formed a looser ("dialytic") staminal cone and had only 10% the fruit set of the individuals with normally pubescent anthers, even though fully self-compatible. The mutant is more like the species of *Solanum* from which *Lycopersicon* presumably arose. Rick speculated that the acquisition of the hairs yielded the tighter anther cone and with it regular selfing in tomatoes.

This may have promoted rapid differentiation of the *Lycopersicon* complex from *Solanum* (Rick, 1947).

Zavada (1984, 1990) argued, but not without challenge (e.g., Gibbs and Ferguson, 1987), that pollen morphology is strongly correlated with the compatibility system of species of angiosperms at the broadest levels. Zavada suggested that reticulate-perforate exine sculpturing is correlated with sporophytic self-incompatibility, and that imperforate and microperforate exine sculpturing is correlated with gametophytic self-incompatibility. This study was done to understand the compatibility structure of paleo-pollen, but if Zavada is correct, we have available a set of palynological features that contain significant clues to the breeding systems of extant taxa as well.

6. Correlated character changes can lead to dramatic phyletic changes—Anagenesis in lineages.

An awareness of the extent to which whole suites of characters might change with acquisition of a new reproductive system is of importance to systematists in understanding such things as anagenetic changes in lineages. For instance, a switch in a lineage from outcrossing to selfing could involve a whole set of new characters, many perhaps even linked developmentally (e.g., Diggle, 1992), that could make a species look very different from its closest relatives, and perhaps similar to species that independently acquired similar changes (Ornduff, 1969; Wyatt, 1988). This obviously could lead to errors in taxonomy; the autogamous solanums cited above are a good example of this. In a review of development and reproductive characters, Diggle (1992) discusses covariation at some length, pointing out developmental correlations not only among floral parts but also between floral and vegetative structures. For instance, an increase in the size of one structure, like leaves, may be associated with an increase in the size of others, like flower parts; the latter could obviously have ramifications for pollination biology (Diggle, 1992).

Also, switches in gamete distribution syndromes—pollination (and dispersal)—can have dramatic effects. As indicated above, many biologists from Darwin on (e.g., Stebbins, 1947; Grant, 1949; Takhtajan, 1969; Basinger and Dilcher, 1984; Crepet and Friis, 1987) have argued convincingly that the acquisition of biotic pollen distribution was the key, possibly to the origin of angiosperms, but very likely to their diversification, beginning in the late Mesozoic and continuing to the present.

The impact of a "reproductive revolution" could be even greater if the new breeding system involves some sort of floral unisexuality. Ornduff (1969: 126) was not convinced of this when he said, "Such separation of the sexes (particularly dioecism) does not usually cause taxonomic problems." He is probably correct in that the majority of such species, at least eventually, have not presented taxonomic problems. But several examples show that changes in reproductive biology can sometimes have broad systematic implications. I will discuss three.

Quinn (1991 and pers. comm.) reports that the effect of dioecy on taxonomy of grasses has been two-fold. First, the dimorphism of staminate and pistillate plants of such species as *Buchloe dactyloides* (Nutt.) Engelm. (buffalo grass) was so striking that they were originally treated as separate genera. This illustrates the kind of mistake that is not uncommon (see below) in taxonomic treatments, mistakes resulting from a lack of appreciation for the ramifications of reproductive changes. An interesting secondary point is that the marked modification of the pistillate inflorescence in sexually differentiated grasses appears to be associated primarily with dispersal (Davidse, 1987). Quinn also notes (pers. comm.) that in grasses major changes in reproductive biology (such as dioecy) often lead to an overemphasis, rather than underrecognition, of differences among species, and lead to taxonomic splitting at the generic level. For example, two species of *Bouteloua* were moved

to different genera when recognized as dioecious. Also, buffalo grass and four close relatives in Mexico (the "Buchloe group"), all dioecious, are placed in five monospecific genera (Quinn, pers. comm.).

Romero's studies of orchids (Romero, 1992) provide some dramatic examples. These orchids were earlier studied by Charles Darwin. Darwin began a paper in 1862 as follows: "The President and Officers of the Linnaean Society having kindly permitted me to examine the remarkable specimen, preserved in spirits in their collection, of an Orchid bearing flowers of two supposed genera, and known sometimes to bear the flowers of a third genus" (C. Darwin, 1862: 151). Darwin (1862) concluded that the three genera (*Catasetum, Monachanthus, Myanthus*) were unjustified and recognized the flowers as three sexual morphs of *Catasetum*. He reached this conclusion in spite of the fact that he considered these "sexual forms" as "...much more different than, for instance, the peacock is from the peahen" (1862: 157). Romero (1992) later clarified that the third hermaphroditic morph is mostly sterile, and that maleness (staminate flowers) and femaleness (pistillate flowers) are mutually exclusive in the flowers.

Although Romero (1992) estimates that the vast majority of orchids (more than 95%) bear hermaphroditic flowers, the differences in floral type in those that have unisexual flowers (e.g., *Catasetum, Cycnoches*, *Mormodes*, and *Satyrium*) can be dramatic, even spectacular (see photographs in Romero, 1991). Romero shows clearly that the differences between the staminate and pistillate flowers of these genera are so great that it is hard to accept that they are simply sex forms. It is these kinds of cases that so clearly make the point that reproductive biology can have a major effect on morphological expression, and thus, on determination of species limits. Presumably this variation is a manifestation of the pollination system.

Another, but less dramatic, example comes from work I have been involved with in *Solanum*. Correll (1962) recognized three species of *Solanum* from Mexico and Central America. All three species bear morphologically hermaphroditic flowers, with pollen-bearing anthers and ovule-bearing ovaries. However, the pollen of two of the "species," though highly fertile (stainable), is unusual in being inaperturate vs. the typical tricolporate pollen for *Solanum* (Anderson and Gensel, 1976; Levine and Anderson, 1986). A series of studies have shown that the three species are morphologically hermaphroditic but functionally, and cryptically, dioecious; they constitute simply the sex forms, the staminate and pistillate flowers of a single species, *S. appendiculatum* Dunal. The pistils of the functionally staminate flowers bear ovules, but pollination never results in fruits or seeds (Anderson, 1979; Anderson and Levine, 1982). The pollen in the anthers of the functionally pistillate flowers is not only morphologically distinct (inaperturate) but also functionally different. The inaperturate pollen, though similar to the tricolporate in stainability, weight, and constituents, cannot be induced to germinate under any conditions *in vitro* or *in vivo* (Levine and Anderson, 1986). There is also a significantly smaller quantity of inaperturate pollen in the pistillate flowers than there is tricolporate pollen in the staminate flowers. The inaperturate pollen is important in pollination in spite of the fact that it plays no role in fertilization (Anderson and Levine, 1982), because it serves as the reward to pollen-collecting pollinators (*Solanum* flowers bear no floral nectar; Anderson and Symon, 1985). This sort of systematic misjudgment, recognizing three species where there is only one sexually dimorphic taxon, puts Correll (1962) in good company. According to Mayr (1963: 32), Linnaeus originally recognized male and female mallard ducks as separate species (*Anas bochas*—male; *Anas platyrhynchos*—female).

The important points from this study and subsequent work on 21 Australian monoecious and dioecious species (Anderson and Symon, 1988a, 1988b) are that a large

number of floral traits are more important as keys to understanding the reproductive biology of the species than they are as guideposts to the systematics. Or, viewed another way, knowledge of the reproductive biology is essential to proper interpretation of the characters. Of particular interest is the fact that a number of features have evolved in common between very distinct lineages. *Solanum appendiculatum* is placed in a subgenus (*Solanum*) divergent from that of the Australian species (subgenus *Leptostemonum*) (Anderson and Symon, 1988b). Thus the common reproductive traits between these groups represent homoplasious characters that reflect evolution in breeding systems, not synapomorphies that denote the phylogeny, and their independent origin is perhaps indicative of the way the genus *Solanum* responds to certain ecological/evolutionary pressures.

CONCLUSIONS

As the textbooks in plant systematics show, the importance of reproductive biology in shaping features at the species level and below is well accepted. Furthermore, there is recognition that angiosperm diversification probably came about in large part because of acquisition of biotic pollen-distribution syndromes. It is also generally accepted that in many individual cases character changes in such features as pollen exine, stigma size, flower size, etc., have an important impact on systematic treatments; but these character changes may be more significant for elucidating breeding systems than systematic relationships. Given all these, it seems clear that the contributions of reproductive biology *sensu lato* to shaping the characters of systematic importance must be acknowledged at all levels—from populations through species and genera to families, orders, and classification at the highest levels among angiosperms. Features of the reproductive biology of plants are important in and of themselves for understanding mechanisms of evolution, and for proper interpretation of characters utilized in systematics.

LITERATURE CITED

Anderson, G. J. 1979. Dioecious *Solanum* of hermaphroditic origin is an example of a broad convergence. Nature 282: 836–838.

_____ and P. G. Gensel. 1976. Pollen morphology and the systematics of *Solanum* section *Basarthrum*. Pollen & Spores 18: 533–552.

_____ and D. A. Levine. 1982. Three taxa constitute the sexes of a single dioecious species of *Solanum*. Taxon 31: 667–672.

_____ and D. E. Symon. 1985. Extrafloral nectaries in *Solanum*. Biotropica 17: 40–45.

_____ and _____. 1988a. Insect foragers on *Solanum* in Australia. Ann. Missouri Bot. Gard. 75: 842–852.

_____ and _____. 1988b. Functional dioecy and andromonoecy in *Solanum*. Evolution 43: 204–219.

Baker, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9: 347–349.

Basinger, J. F. and D. L. Dilcher. 1984. Ancient bisexual flowers. Science 224: 511–513.

Brown, A. D. H. and J. D. Briggs. 1991. Sampling strategies for genetic variation in *ex situ* collections of endangered plant species. Pp. 99–119 *in* D. A. Falk and K. E. Holsinger (eds.), Genetics and Conservation of Rare Plants. Oxford Univ. Press, New York.

Correll, D. S. 1962. The Potato and Its Wild Relatives. Texas Research Foundation, Penner, TX.

Crepet, W. L. and E. M. Friis. 1987. The evolution of insect pollination in angiosperms. Pp. 181–201 *in* E. M. Friis, W. G. Chaloner and P. R. Crane (eds.), The Origin of Angiosperms and Their Biological Consequences. Cambridge, New York.

Cruden, R. W. 1977. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

Darwin, C. 1862. On the three remarkable sexual forms of *Catasetum tridentatum*, an orchid in the possession of the Linnean Society. J. Proc. Linn. Soc., Bot. 6: 151–157.

Davidse, G. 1987. Fruit dispersal in the Poaceae. Pp. 143–155 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth (eds.), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

Davis, P. H. and V. H. Heywood. 1963. Principles of Angiosperm Taxonomy. Van Nostrand, Princeton, NJ.

Diggle, P. K. 1992. Development and the evolution of plant reproductive characters. Pp. 326–355 *in* R. Wyatt (ed.), Ecology and Evolution of Plant Reproduction: New Approaches. Chapman and Hall, New York.

East, E. M. and A. J. Mangelsdorf. 1925. A new interpretation of the hereditary behavior of self-sterile plants. Proc. Natl. Acad. U.S.A. 11: 166–171.

Ehrlich, P. and P. Raven. 1969. Differentiation of populations. Science 165: 1228–1232.

Gibbs, P. E. and I. K. Ferguson. 1987. Correlations between pollen exine sculpturing and angiosperm self-incompatibility systems—A reply. Plant Syst. Evol. 157: 143–159.

Grant, V. 1949. Pollination systems as isolating mechanisms in angiosperms. Evolution 3: 82–97.

Hamrick, J. L. 1987. Gene flow and distribution of genetic variation in plant populations. Pp. 53–67 *in* K. Urbanska (ed.), Differentiation Patterns in Higher Plants. Academic Press, New York.

_____ and M. J. W. Godt. 1990. Allozyme diversity in plant species. Pp. 43–63 *in* A. D. H. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir (eds.), Plant Population Genetics, Breeding, and Genetic Resources. Sinauer, Sunderland, MA.

Holsinger, K. E. 1991. Conservation of genetic diversity in rare and endangered plants. Pp. 626–633 *in* E. C. Dudley (ed.), The Unity of Evolutionary Biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology. Dioscorides Press, Portland, OR.

Jones, S. B. and A. E. Luchsinger. 1986. Plant Systematics. McGraw-Hill, New York.

Les, D. H., J. A. Reinartz, and E. J. Esselman. 1991. Genetic consequences of rarity in *Aster furcatus* (Asteraceae), a threatened, self-incompatible plant. Evolution 45: 1641–1650.

Levin, D. 1978. The origin of isolating mechanisms in flowering plants. Evol. Biol. 11: 185–317.

Levine, D. A. and G. J. Anderson. 1986. Evolution of dioecy in an American *Solanum*. Pp. 264–273 *in* W. G. D'Arcy (ed.), Solanaceae: Biology and Systematics. Columbia, New York.

Lewis, D. and L. K. Crowe. 1958. Unilateral interspecific incompatibility in flowering plants. Heredity 12: 233–256.

Lloyd, D. G. 1965. Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). Contr. Gray Herb. 195: 1–134.

Lu, J., A. Mayer, and B. Pickersgill. 1990. Stigma morphology and pollination in Arachis L. (Leguminosae). Ann. Bot. 66: 73–82.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press, Cambridge, MA.

Mione, T. 1992. Systematics and Evolution of *Jaltomata*. (Solanaceae). Ph.D. Thesis, Univ. of Connecticut, Storrs.

_____ and G. J. Anderson. 1992. Pollen-ovule ratios and breeding system evolution in *Solanum* section *Basarthrum*. Amer. J. Bot. 79: 279–287.

Nettancourt, D. de. 1977. Incompatibility in Angiosperms. Springer-Verlag, Berlin.

Olmstead, R. 1989. Phylogeny, phenotypic evolution, and biogeography of the *Scutellaria augustifolia* complex (Lamiaceae): Inference from morphological and molecular data. Syst. Bot. 14: 320–338.

Ornduff, R. 1969. Reproductive biology in relation to systematics. Taxon 18: 121–133.

Pandey, K. K. 1962. Interspecific incompatibility in *Solanum* species. Amer. J. Bot. 49: 874–882.

Philbrick, C. T. 1984. Pollen tube growth within vegetative tissues in *Callitriche* (Callitrichaceae). Amer. J. Bot. 71: 882–886.

Philbrick, C. T. and G. J. Anderson. 1987. Implications of pollen/ovule ratios and pollen size for the reproductive biology of *Potamogeton* and autogamy in aquatic angiosperms. Syst. Bot. 2: 98–105.

Quinn, J. A. 1991. Evolution of dioecy in *Buchloe dactyloides* (Gramineae): Tests for sex-specific vegetative characters, ecological differences, and sexual niche-partitioning. Amer. J. Bot. 78: 481–488.

Rick, C. 1947. Partial suppression of hair development indirectly affecting fruitfulness and the proportion of cross-pollination in a tomato mutant. Amer. Naturalist 81: 185–202.

Romero, G. A. 1991. Orquídeas unisexuales. Invest. Ci. 172: 66–75.

_____. 1992. Non-functional flowers in *Catasetum* orchids (Catasetinae, Orchidaceae). Bot. J. Linn. Soc. 109: 305–313.

Schoen, D. J. and A. D. H. Brown. 1991. Intraspecific variation in population gene diversity and effective population size correlated with the mating system in plants. Proc. Natl. Acad. U.S.A. 88: 4494–4497.

Stebbins, G. L. 1947. Evidence on rates of evolution from the distribution of existing and fossil plant species. Ecol. Monogr. 17: 149–158.

Stuessy, T. F. 1990. Plant Taxonomy. Columbia, New York.

Takhtajan, A. 1969. Flowering Plants. Smithsonian Institution Press, Washington, D.C.

Whalen, M. D. and G. J. Anderson. 1981. Distribution of gametophytic self-incompatibility and infrageneric classification in *Solanum*. Taxon 30: 761–767.

Wyatt, R. 1988. Phylogenetic aspects of the evolution of self-pollination. Pp. 109–131 *in* L. D. Gottlieb and S. K. Jain (eds.), Plant Evolutionary Biology. Chapman and Hall, London.

Zavada, M. S. 1984. The relation between pollen exine sculpturing and self-incompatibility mechanisms. Pl. Syst. Evol. 147: 63–78.

_____. 1990. Correlations between pollen exine sculpturing and angiosperm self-incompatibility systems—A rebuttal. Taxon 39: 442–447.

PHYLOGENETIC HISTORIES WITHIN AND AMONG SPECIES[1]

Wayne Maddison[2]

ABSTRACT. Genes have gene trees, just as species have species trees. That is, the copies of a gene from different individuals within a species will be related to one another by a branching pattern of descent analogous to the branching of species lineages in the phylogenetic tree of life. This holds as long as the gene has not suffered recombination within it during the time under consideration. If it has, then the gene's history will be broken up into a series of trees, one for each unbroken region of the gene. Many processes of population genetics can be viewed as relating to the shapes and relationships among gene trees, and an explicit consideration of gene phylogenies stands to help our understanding of gene flow, effective population size, and other population genetics parameters. Phylogenies of species themselves are also relevant to population biology, for if the ideas of population biology are to relate to long-term evolution, they must predict (and hence be tested by) patterns at broad phylogenetic scales.

A gene's trees in different species eventually connect into a large interspecific gene tree. Because the branching of the gene tree can extend deeper than the branching between the species, the relationships of gene copies might not reflect the relationships of the species containing them. This possible discordance should make us reconsider what in fact are species phylogenies. A species phylogeny might be viewed as purely a history of genetic connections, or as a model of the breakup of potential interbreeding. In the former view, the species phylogeny is more like a statistical distribution, being composed of various trees (the gene trees), each of which may indicate different relationships.

My purpose in this paper is to build in your mind a strong image of the trees of genetic descent, as they occur within species and among species. We are all familiar with the notion of a phylogenetic tree among species, depicting the pattern of speciation and the descent of species, but less familiar may be the notion of trees within species. These within-species "phylogenies" are the histories of descent of genes. My first task will be to review some simple facts to emphasize that genetic lineages have a tree-like history within species. Then, I will summarize a few recent results that indicate why it may be important for population biologists to consider these tree-like genetic histories, and for phylogenetic biologists to consider genetic processes within populations.

TREES WITHIN POPULATIONS

What does a population look like in your mind's eye? In mine it looks something like Figure 1, at least if the species is sexual, diploid and outcrossing. The current generation, in 1992, is represented in the top row. Each oval represents one diploid individual. This is an admittedly small population, with only 10 individuals in the current generation, but it will serve to illustrate some general points. As one looks down in the figure, one looks back to previous generations.

[1]I thank Michael Donoghue and Peter Hoch for the opportunity to speak. Leticia Avilés, Brent Burt, Alan de Queiroz, Michael Donoghue, Joe Felsenstein, Richard Hudson, Junhyong Kim, David Maddison, and Kerry Shaw provided valuable discussion and comments.

[2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, U.S.A.

Each individual has two parents, and may give rise to one or more offspring. These parent-offspring relations are shown in Figure 1 by lines connecting the ovals in successive generations. Thus, the connections among individuals in a sexual population do not form a simple branching tree, because the lines both branch and anastomose. The diagram of genetic connections is therefore highly reticulate. Indeed when many people think about doing phylogenetic work below the species level, they respond that it can't be done, because the history is not one of trees. After all, how could one talk of synapomorphies delimiting clades when there are no clear clades? Such concerns about reticulate histories also arise at higher levels with some organisms, such as some plants, suspected of having rampant hybridization between species.

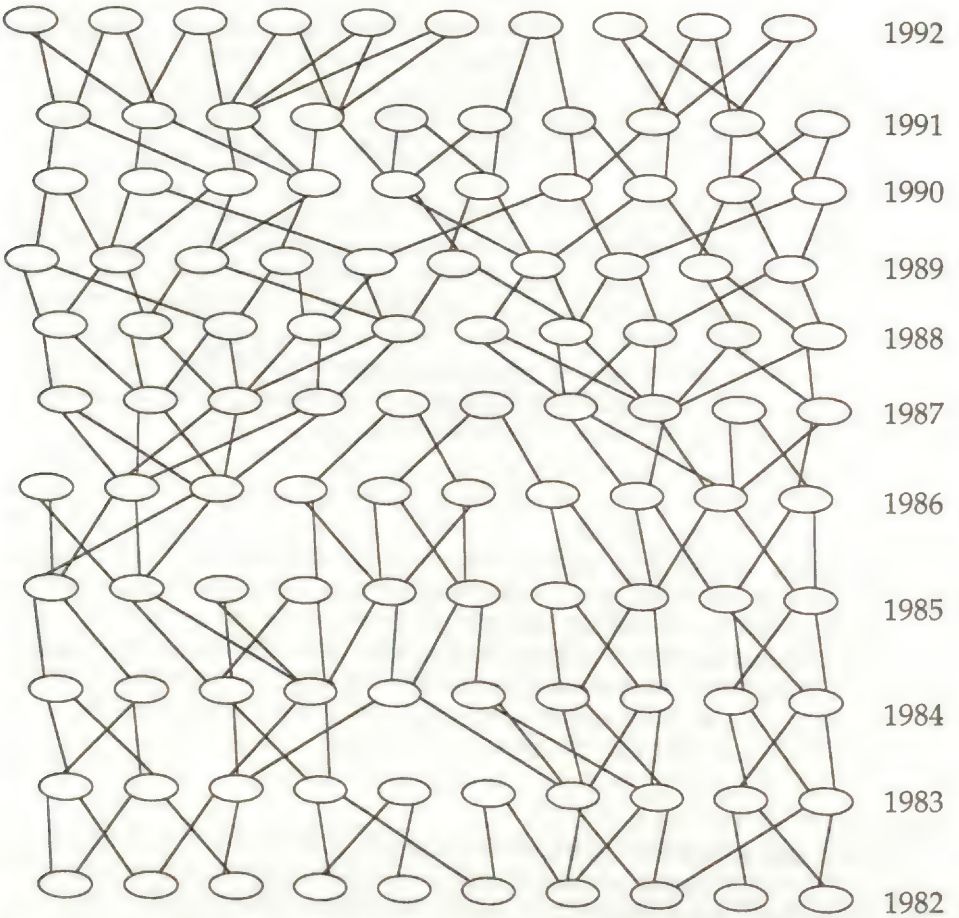


Figure 1. Parent-offspring connections in small diploid annual population over the last 11 generations. Each oval represents a single individual; note that each has two parents.

However, the point of this paper is that there are trees everywhere, even in the population of Figure 1. Let's follow the history of a particular gene. By "gene" I mean a region of the nuclear genome small enough that no recombination has happened within it. That is, I am using the word "gene" in the sense of a unit of recombination.

The history of one gene's transmission through the population might be represented by a diagram like that in Figure 2. Each diploid individual has two copies of the gene, repre-

sented by two small circles, one of which came from one parent, one from the other parent. In the 10 individuals in the 1982 generation of this population, there were 20 copies of the gene. Let's follow the fate of some of these copies, which are labelled 1 through 20 in Figure 3.

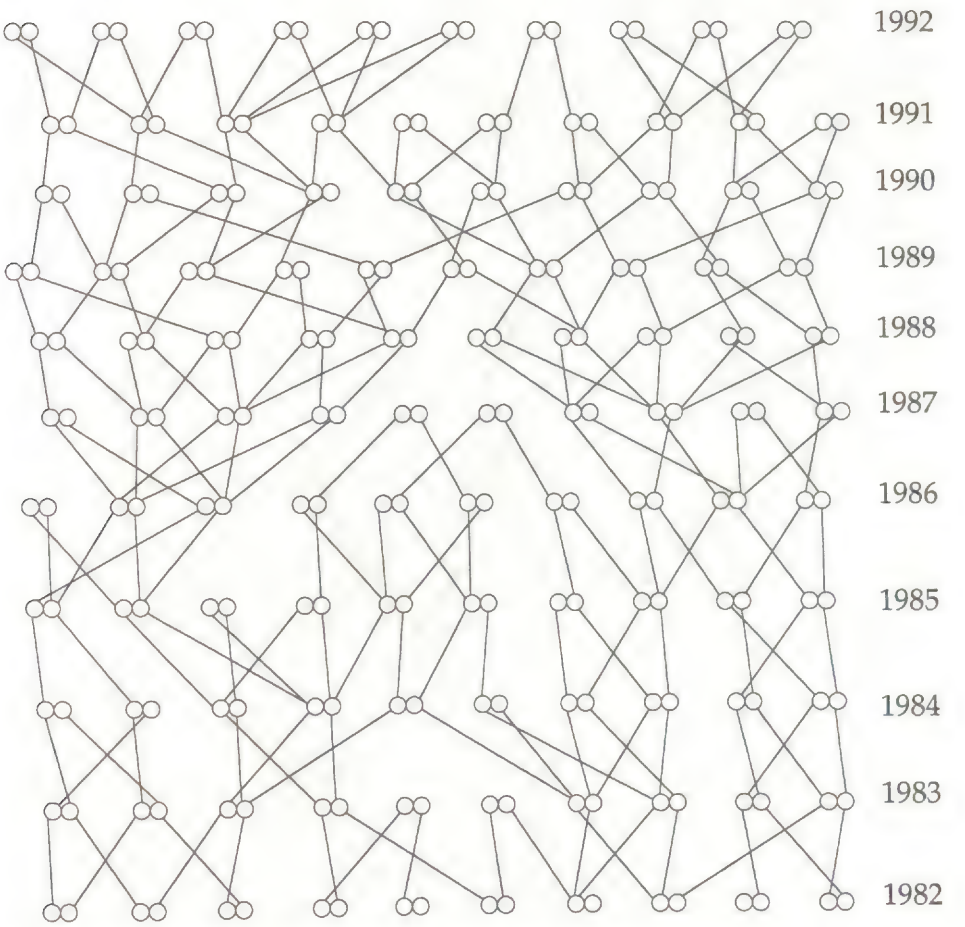


Figure 2. The history of descent of copies of a single nuclear gene in the same population as Figure 1. Each individual is now represented by two circles, one for each of its copies of the gene. Note that one of the copies comes from the individual's mother, one from its father.

Figure 3 highlights the fates of four of the copies. Copy number 4 left one descendant copy in the 1983 generation, in the next, one copy, but in the following generation left two copies. If we follow this gene copy's descendants further we see it proliferates well and leaves many survivors in the current generation. The form of its history is a tree, that is, a strictly branching history. It looks here and there as if there has been some reticulation, but that is just because in my figure the branches happen to cross. History was not so kind to the other three copies highlighted in Figure 3, and they left no surviving descendants. Indeed, of the 20 copies in the 1982 generation, 15 of them went extinct by 1992. I invented this population and its genetic connections haphazardly, attempting only to ensure that each gene copy had an ancestor. And yet it shows a characteristic expected in natural populations: a lot of ex-

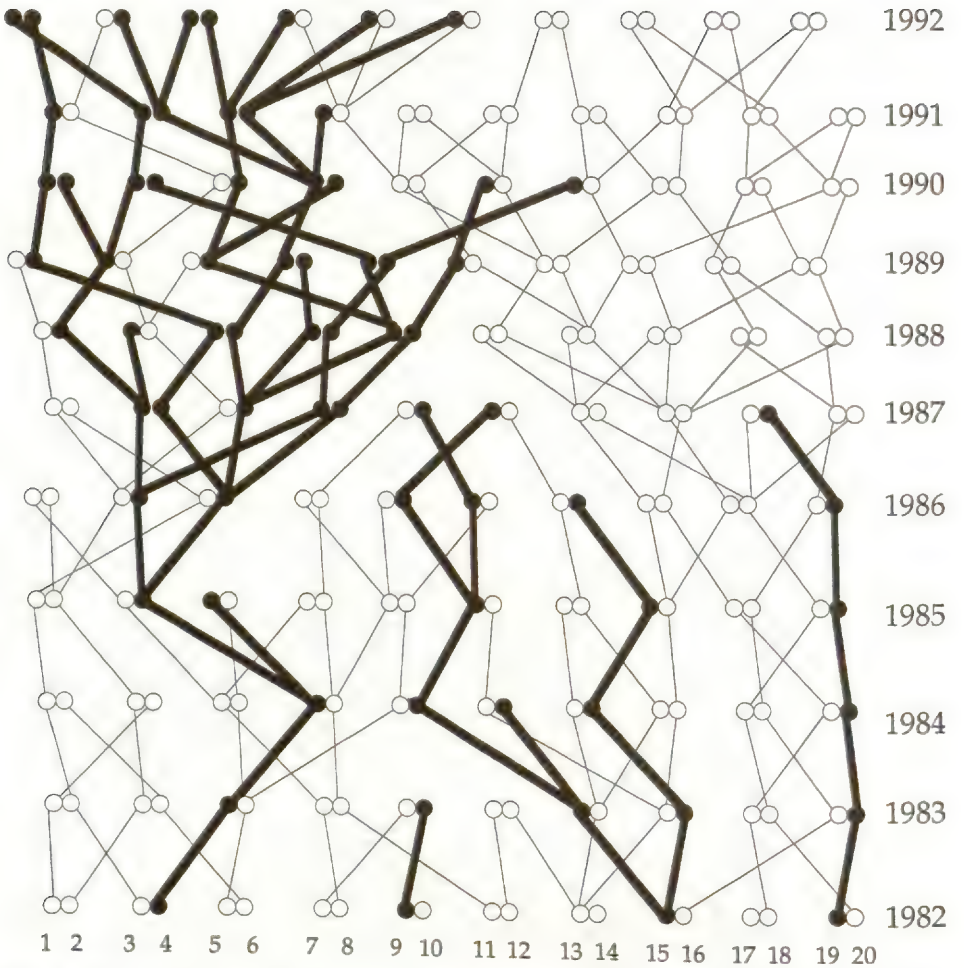


Figure 3. Fates of four copies of the gene that were in the 1982 generation. Each copy in that generation is numbered. The descent of copies 4, 9, 15, and 19 is highlighted.

tinction of copies of genes. Extinction of gene copies is a constant and normal phenomenon, and yet it has consequences that may seem surprising. For instance, the fact that all extant human mitochondria may descend from a single female in the last few hundred thousand years (Cann et al., 1987) would be a simple consequence of the extinction of the mitochondrial genes of all of her contemporaries.

If one were to follow every one of the 20 copies of 1982, in every case the form of the gene's history is a tree. The reason is simple: every copy has only one ancestor, and it may have one or more descendants. (A gene could have more than one ancestor if it is a recent recombinant, but remember that I am using the term "gene" to refer to a section of the genome within which recombination has not occurred in the scope of the history being considered.) These trees are sometimes called gene trees, or gene genealogies, or gene phylogenies. Of course, in many cases these trees will not be reconstructible, if no mutations have occurred to mark their branches. But whether or not discoverable, the pattern of descent of gene copies must have been tree-like.

I have talked about the gene as if it has 20 separate gene trees, one arising from each of the original copies in the 1982 generation. However, it is actually best to think of the gene figured as having a single large tree, because if we look back far enough before 1982, there would exist a single common ancestor of all of the 20 copies in the 1982 generation, and thus each of their 20 trees, of which a few are highlighted in Figure 3, would coalesce into a single common tree.

I will not try to draw a figure showing how all of the 1982 copies coalesce into a single common ancestor, because the figure would be too long. However, I will show the ancestry of all of the extant copies (1992) and show how their history takes the form of a single tree. Figure 4 begins with the current copies and looks backward. As can be seen, historical relationships of these copies take the form of a tree, whose root or common ancestor occurred in the 1979 generation. The same can be said for any sample of gene copies in this population: they must be related to one another by a single gene tree.

The apparent non-treeness of the history of the population in Figure 1 comes from two facts. First, the tree of different copies of an individual gene is folded over itself, so that its branches find each other momentarily sharing residence in individual diploid organisms, as seen in Figures 2 and 3. Second, different genes will have different histories, if they are far enough away from one another in the genome that recombination has occurred between them. (Gene conversion can add additional complications that I will not consider here.)

That different genes in the genome can have different histories can be seen by considering two nearby genes. Let us call the original gene, the one we have been discussing so far, "gene A." Consider a nearby gene, gene B, so near to gene A in the genome that in this short history of 11 generations only one recombination event has occurred between the two gene loci. In gene A, let us focus on the descendants of copies 4 and 13 of the 1982 generation. These are shown in Figure 5; the descendants of copy 4 are shown in gray, those of 13 in black.

Now let us consider gene B. Suppose there was a recombination event, between genes A and B, in the gametogenesis of the individual marked by an oval in Figure 5. Because of the recombination event, the gamete that the marked individual donated to one of its 1991 offspring will differ in the grandparental source of genes A and B. If there had been no recombination, the individual with the oval would have placed in the gamete its father's allele (for example) of both of these gene loci. However, there was recombination, and the individual instead placed its father's allele of gene A and its mother's allele of gene B. As can be seen in Figure 5 and as was realized by Hein (1990), the effect of a single recombination event is to make the gene trees of two genes differ by a single branch move. In this example, there is a branch of gene A's tree that attaches to the black clade (a descendant of copy 13) and belongs to the black clade; in gene B's tree it arises out of the gray clade. The histories of the two nearby genes are slightly different. Both histories take the form of a tree, but the two trees differ in the position of a branch.

What if we were to look at a gene that was not nearby in the genome, but rather completely unlinked to the first? It is constrained to have its pathways of descent follow through the same parent-offspring links, depicted in Figure 1, as do genes A and B, but the exact descent of gene copies can differ substantially. In Figure 6 are close-up views of parts of the gene histories in this population. At the top of the figure is gene A, below is an unlinked gene. Note that each individual has the same parents, but which grandparental genes were inherited differs in many cases. This means that the two genes could have rather different gene trees. For instance, the root of each gene's tree, that is the gene copy that was the most recent common ancestor of all extant copies, would most likely have occurred in different individuals for the different genes. Each gene in the genome likely had a separate "Adam" or "Eve."

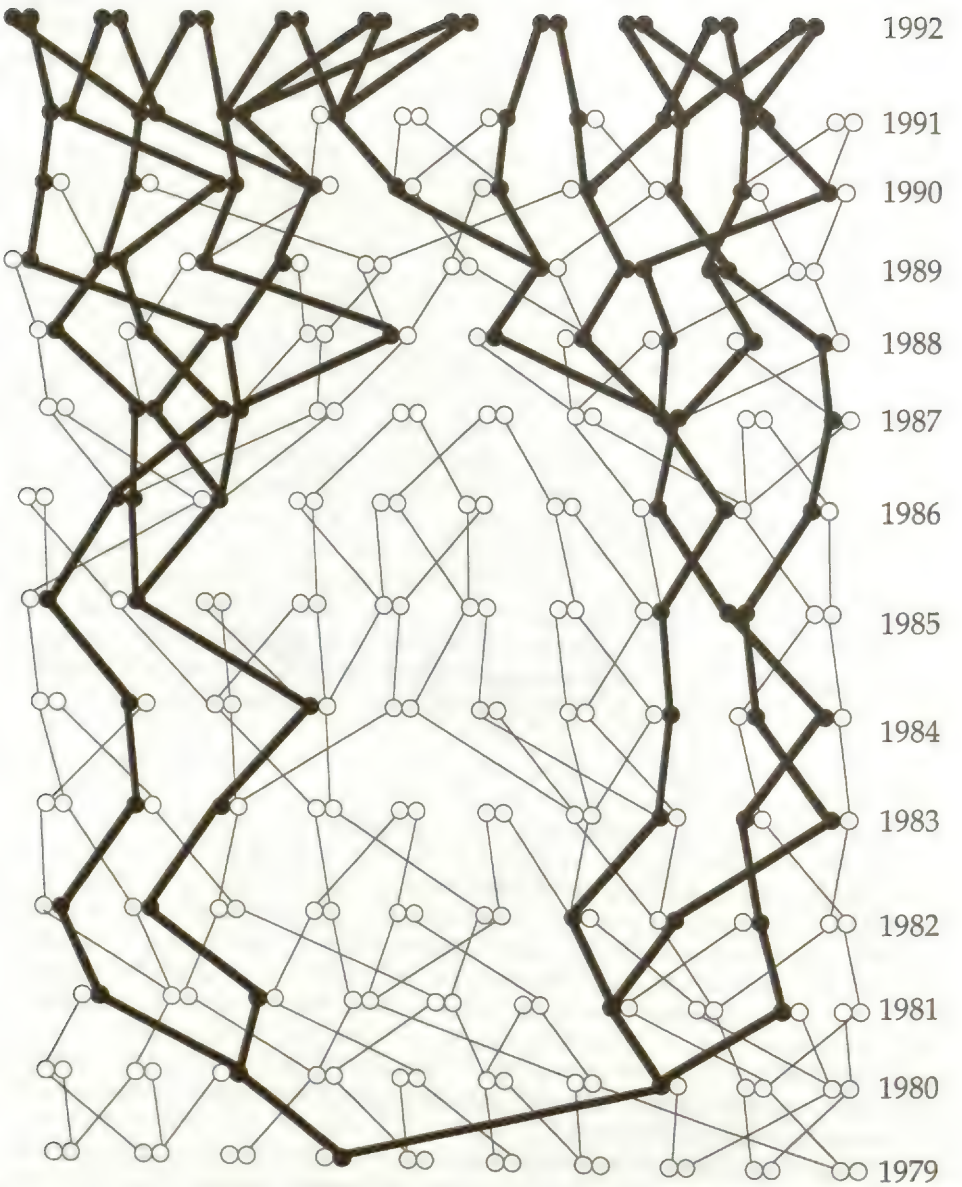


Figure 4. Ancestry of all extant (1992) copies of the gene.

The message behind all this discussion is that sex, recombination and hybridization do not destroy the treeness of genetic history. These processes only divide the genetic history into multiple trees.

I have approached the issue of recombination differently than might a population geneticist. I began with the simple case of a section of the genome that was fully linked (no recombination within), and eventually arrived at the complex case of no linkage. Often in population genetics, independent assortment is the default assumption, simplest to conceive

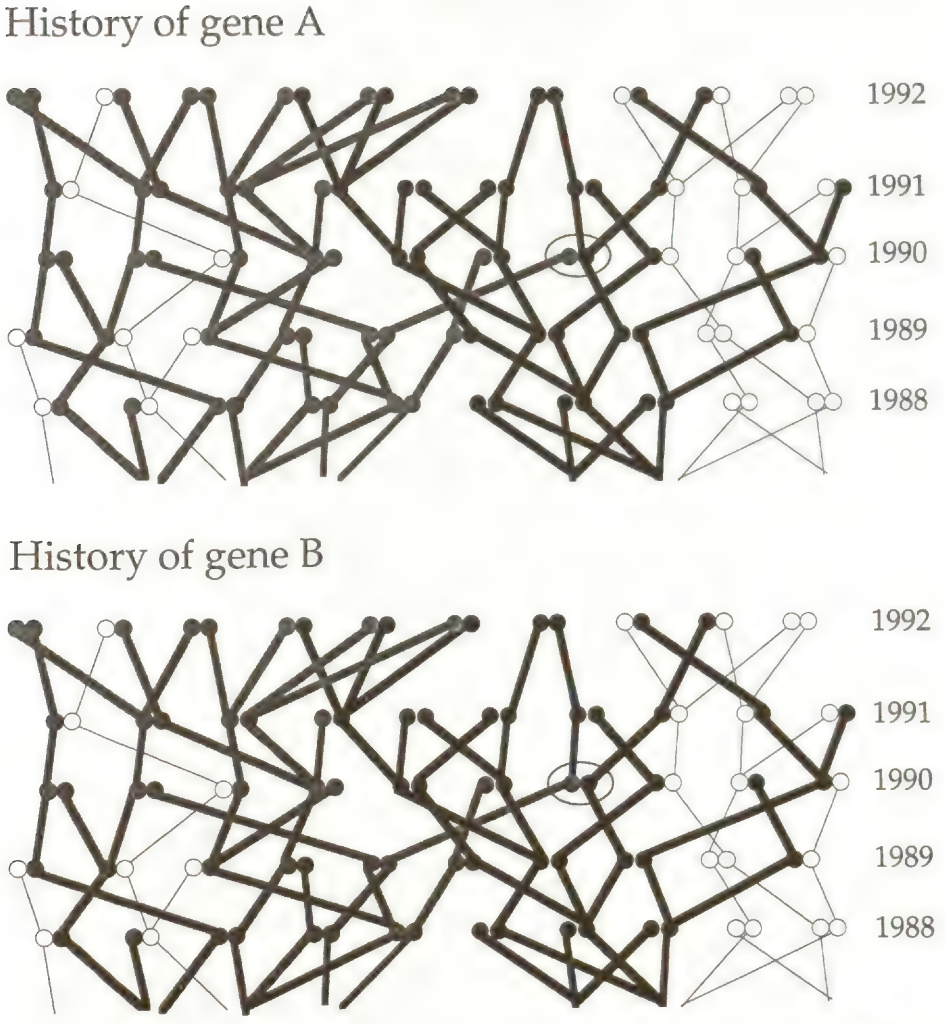


Figure 5. Histories of two nearby genes. In both, the descendants of the 1982 copies number 4 and 13 are highlighted, in gray and black respectively. A recombination event occurred between the two genes in the individual marked with an oval.

and with which to do calculations, while linkage of two or more genes is a complicating factor in calculations and interpretations. In the gene-tree view the opposite is true: full linkage of a series of genes is the simple default assumption, since it leads to a single gene tree, while recombination leads to multiple gene trees.

A NEW DEFINITION OF POPULATION GENETICS

This leads me to suggest a new definition of population genetics:

> *Population genetics* is the study of gene trees in a population, the evolutionary forces that shape them, and the nature of gene changes along their branches.

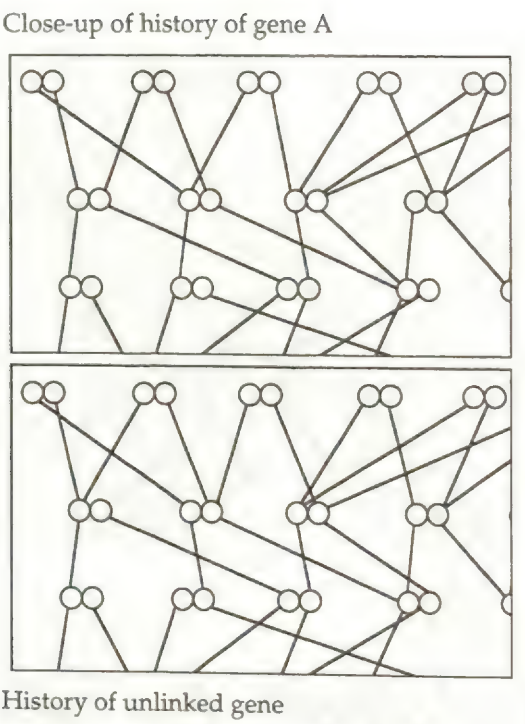


Figure 6. History of two unlinked genes.

By this perspective, population genetics would include study of how gene replication and extinction affect the branching patterns in gene trees (fixation times, drift, selection), how changes in the genes occur along the branches of these trees (mutation), how the tree of a gene folds over itself into individuals (population structuring, inbreeding), how the gene trees relate to geographic location (migration, population differentiation), and how the trees of different genes differ from one another (recombination). Any population genetics process might leave its mark in the shape, branch lengths, and other characteristics of the species' gene trees.

This definition is not presented as a replacement for other concepts of population genetics. Rather, it yields an alternative perspective, one to be explored to see how far it will lead us. The forces behind these gene trees are the forces behind population genetics, and we might at least consider how a phylogenetic view of genes could help us think about population biology. This gene-tree view of population genetics is by no means original to me. Population geneticists for many years have implicitly considered the replication and extinction of genes. And now, the new field of coalescent theory devotes itself explicitly to considering gene trees and populations by looking backward in time at the branching histories of genes (see Hudson, 1990, for a review).

THE VALUE OF A PHYLOGENETIC PERSPECTIVE

One question you may have is why does one need to consider the exact branching history of the genes? Physicists are quite happy with laws like the gas laws that describe the mass behavior of many gas molecules without worrying about their individual histories. Do population geneticists need to consider the individual histories of genes, or can they be treated like gas molecules?

Gene flow. The first thing that might be gained from the individual histories of genes is an alternative means to estimate population genetic parameters. Slatkin and Maddison (1989, 1990) and Hudson et al. (1992) began investigating the value of gene trees in estimating the rates of gene flow. Their method works as follows. Suppose gene copies have been sampled from individuals in various localities, in this case let's say east and west (Figure 7), then the phylogenetic interrelationships of the gene copies are reconstructed. The degree to which the east and west gene copies are intermixed on the phylogeny should tell something about the amount of gene flow between the localities. For instance, if the phylogeny were as in the left gene tree of Figure 7 then it would seem as if there has been little migration of genes between east and west. On the other hand, if the phylogeny were as in right gene tree of Figure 7 it would seem as if more migration occurred. Indeed, standard cladistic techniques show that at least 5 migration events must have occurred. Slatkin and Maddison (1989) chose to use this minimum number of migration events required, called "s," as an estimator. It turns out that for a panmictic population without selection the probability distribution of s can be calculated exactly (Maddison and Slatkin, 1991), allowing a test of the null hypothesis that the two localities are part of the same panmictic population. Simulations were required to derive expected distributions of s under various levels of gene flow (Slatkin and Maddison, 1989) and s was compared to other gene flow measures under various levels of recombination (Hudson et al., 1992). Their results show that the statistic s is a reasonably good estimator of gene flow, although in many situations it would have to be calculated for several independent gene loci to yield a better estimate than those from other methods available.

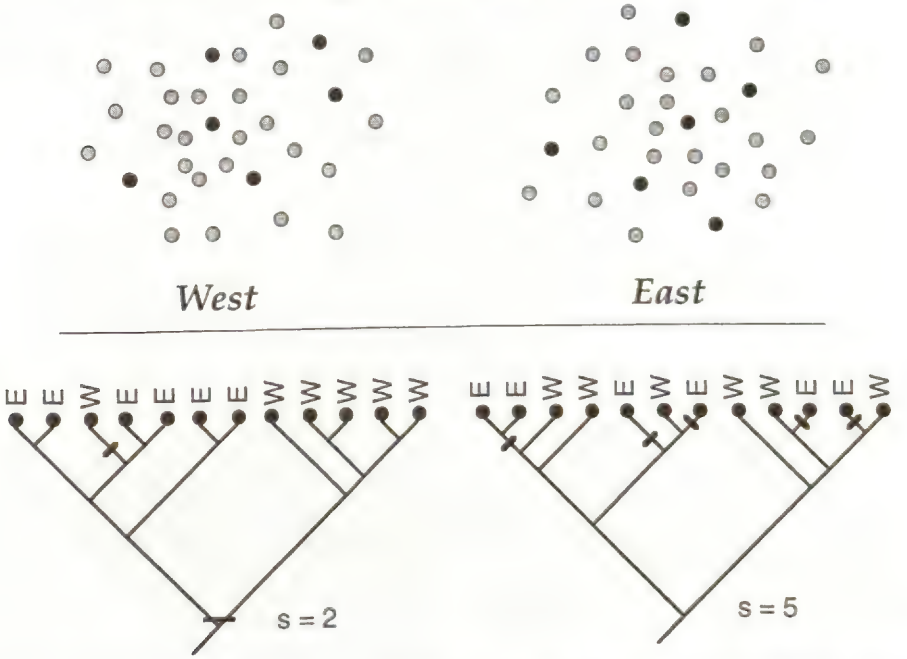


Figure 7. Genes (represented by spots) from two localities, west and east, and two possible gene trees relating the 12 genes sampled. Above, black spots represent the genes that were sampled. The left gene tree might be expected if there had been little gene flow between east and west, the right gene tree if there had been much gene flow.

Effective population size. There are some population questions for which a phylogenetic method can tell us more than methods that fail to account for gene histories. For in-

stance, consider the problem of estimating effective population size. Let us suppose we have a population that has had a constant population size, and we sample gene copies from it. There is a simple relationship between the population size and the expected ages of common ancestors of the gene copies. If effective population size has been small, then the most recent common ancestral gene copy was likely found in the recent past, but if population size has been large, the common ancestor was likely found in the more distant past (assuming panmixia and no selection). The degree of difference between nucleotide sequences might therefore be used as an indicator of the age of their ancestor, which in turn could be used as an indicator of effective population size.

Felsenstein (1992a) has shown that a method that uses pairwise sequence differences to estimate effective population size is not as good as a method that takes into account the phylogeny of the sequences. Indeed, as the amount of information (number of sequences) increases, the phylogenetic method yields a better and better estimate, but the pairwise method reaches a plateau and gets no better. The reason for the pairwise method's failure is as follows. In an average gene phylogeny at least 1/3 of the pairwise comparisons are between two sequences that are on either side of the root of the gene phylogeny (Felsenstein, 1992a). This means that many of the pairwise comparisons are not independent from one another. Because so many pairs refer to the same node in the tree, there is an amount of variance in the estimate that more sequences can't reduce. This is an illuminating finding. It shows that even within populations, historical interrelationships and statistical dependencies should be considered.

Need for a well-reconstructed phylogeny. Skeptics will be concerned that such approaches require an accurately reconstructed gene phylogeny. This is a valid concern, especially for study of population processes so recent that it will be difficult to find enough genetic changes to reconstruct gene trees. This may mean that a phylogenetic approach will not be very productive. However, perhaps in such circumstances no other approach would be any more productive; some facts may simply be unknowable. To promote a phylogenetic perspective, I might claim that for any population genetics method that ignores gene trees there will always exist a gene tree approach that is at least as good, if not better, since adding a gene-tree perspective can only improve the method. However, such a claim may be misleadingly optimistic, given that the gene tree approach might result in only marginal improvement, and would in practice have to be able to deal adequately with limited data and great uncertainty in the gene tree.

In developing methods based on gene trees, one of our greatest challenges will be to account for uncertainty in the tree. One promising approach has been developed by Felsenstein (1992b). He has worked out a method that integrates over all possible gene trees to derive a maximum likelihood estimator for effective population size. By integrating over all possible gene trees (using coalescent theory to supply a prior probability by which to weight each tree's likelihood), he takes into consideration that a gene tree existed without having to know what particular tree it was. Thinking phylogenetically can be valuable even when the particular phylogeny is not known.

INTERSPECIFIC PHYLOGENIES

So far I have dwelled on trees of genes within species. If you are a population biologist, should you care about phylogenetic trees of species? The answer is yes, for a simple reason. If the processes you study within populations have any relevance at all to long-term evolution, then they should yield traces in the broad-scale patterns of diversity. Thus, across-species patterns can test your notions of within-species process, depending on whether observed patterns across species match what you would expect from your hypothesized processes.

There has been much work recently on a phylogenetic approach to across-species patterns in comparative biology. It has been emphasized many times that it is important to consider phylogeny (e.g., Ridley, 1983; Felsenstein, 1985; Donoghue, 1989; Harvey and Pagel, 1991; Maddison and Maddison, 1992). For instance, if different species are plotted on a scattergram according to their values in two variables of interest, there may be an apparently significant correlation between the two variables. But if it so happens that the cloud of points at the upper right of the scattergram represents the species from a single clade, the cloud at lower left from a second clade, then it is clear that there really isn't strong evidence for a causal connection. In fact, the two variables may have simply changed independently on the same branch of the phylogenetic tree. The co-occurrence of a change in the two variables is observed only once, and a single sample point is surely not convincing of a correlation. The species are not statistically independent sample points because of their greater or lesser amounts of shared histories. If comparative patterns across species are sought, it is absolutely critical that their phylogenetic interrelationships are considered.

If the importance of considering phylogeny and historical dependencies is well-accepted across species, should it also be accepted within species? Perhaps Felsenstein's work on effective population size has given us one reason to suspect it should. We need to ask ourselves: How many other population genetics questions are there for which knowledge of the histories of genes should be an integral part of our thinking? There are many questions for which classical population genetics will continue to be appropriate, but there will be other questions in which an explicitly gene-tree view will be indispensable.

SPECIES TREES VERSUS GENE TREES

After discussing gene phylogenies within species, then phylogenies of species, I would like to come to the final point that the border between population biology and phylogenetic biology of species is not nearly so distinct as it might at first appear.

Imagine there was an ancestral species that split into two. One of the descendants split again. Therefore, the species splitting through time formed a small species phylogeny of three species, which I will call A, B and C, as shown in Figure 8. The straight, thick lines show the boundaries of the species. The thinner, wandering lines show the descent of some gene copies. The species lineages can be pictured as being like tubes through which their genes descend. Three variations on this species phylogeny are shown in Figure 8; they differ in how much time elapsed between the two species-splitting events.

If a copy of a gene is sampled from one individual of each extant species, the ancestors of these three copies must form a small gene tree. You might expect that the two most closely related genes would be those from the two most closely related species, B and C. This would mean that the gene tree of these three genes would match the species tree of the three species containing them. This is what is shown in the left column in Figure 8.

However, the work of Hudson (1983), Tajima (1983), Pamilo and Nei (1988), and Takahata (1989) shows that if the interval between species splitting is short enough, other possibilities are disturbingly common. For instance, if the number of generations between the A-BC split and the B-C split is about half the population size, then in about half of the instances in which three genes were sampled, the gene tree of the three genes would not match the species phylogeny. Examples are shown in the right two columns of Figure 8. In the second column, for instance, the gene sampled from species B is most closely related to the gene sampled from species A, even though C is B's closest relative. When the interval between species splits is longer, then this becomes less and less likely to happen (probability of consistency being 0.76 if the interval in generations is twice the population size, 0.91 if the interval

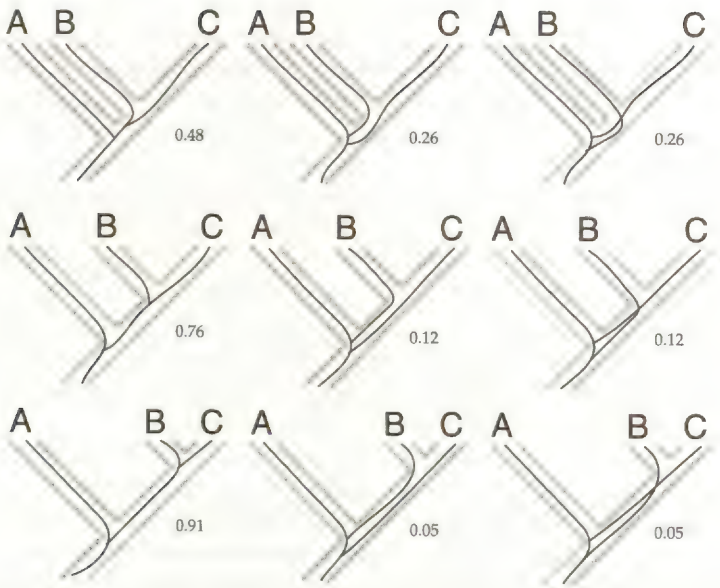


Figure 8. Species trees and contained gene trees. The outer, thick lines represent species, of which three arise (A, B and C) by two speciation events. One copy of a gene is sampled from each of the three species, and the fine lines represent the gene tree relating these three copies. Each row of three trees represents a particular interval between speciation events; in the top row, the two speciation events are 0.5 N generations apart; in the middle row, 2 N generations apart, and in the bottom 4 N generations apart, where N is the diploid population size between the two speciation events. Each column represents a different outcome of gene tree, only the first (left) of which shows the gene tree concordant with the species tree. The probabilities of each outcome are indicated according to the formula of Pamilo and Nei (1988).

is four times the population size). The problems arise when branches are short (measured in generations) compared to their widths (measured in population size).

This problem is distressing for those of us that reconstruct phylogeny. It says that the gene phylogeny might be reconstructed accurately, but it won't be the correct species phylogeny. If the phylogeny is reconstructed based on a different gene or based on different individuals, it might also be reconstructed accurately but be different from that of the first gene locus.

This problem is not restricted to molecular data. For instance, suppose one is trying to reconstruct the phylogeny of different populations or closely related species using phenotypic characters. Imagine that each character has a single controlling gene, and that different characters have genes in different places around the genome. Each of the different characters used could have followed a different true phylogeny. This has long been realized as a possibility for some characters, such as polytene chromosome inversions. But usually phylogenetic biologists assume that different characters followed the same phylogeny and that characters disagree because of convergence. It is disturbing to imagine that characters might disagree because they followed different phylogenies! Polygenic characters do not solve the problem. If a character is controlled by many scattered genes, it may be that its history of changes did not track the branching species phylogeny in any straightforward way, and instead is a muddled history composed of many different gene trees.

I have characterized the problem as a difficulty in reconstructing the species phylogeny, but disagreement among gene trees should make us pause to think about the meaning

and existence of the species phylogeny itself. There has been a great deal of controversy about the nature of species (Simpson, 1961; Mayr, 1963; de Queiroz and Donoghue, 1988; Nixon and Wheeler, 1990; Baum, 1992; Baum and Shaw, 1995), and hence implicitly about the meaning of branching and lineages depicted in a species phylogeny. I do not want to wade too deeply into the turbulent waters of species concepts, but it seems clear that one possible interpretation of a species phylogeny is that it depicts the lines by which genetic information was passed *and nothing more* (e.g., Baum, 1992; Baum and Shaw, 1995). A species phylogeny of this sort, should we know it, would have profound explanatory value with respect to the distribution of the traits of organisms, because the lines of passage of genetic material form among the strongest constraints on the similarities and differences of organisms. Given that we may state the task of evolutionary biology as "explaining why the various organisms are the way they are," a knowledge of the history of the past genetic connections of organisms is vital.

If a species phylogeny depicts only realized genetic descent, then it may be fair to say that a species phylogeny is composed in its entirety of the gene trees of all the individual genes (including the tangling of gene trees through the bodies of individual organisms). If some of those genes have histories that disagree with others, as in Figure 8, then the species phylogeny is a composite of several branching patterns. The species phylogeny is therefore not fully conveyed by the statement "species B and C are more closely related to each other than either is to A." Rather, it might be better conveyed by a statement like "most of the genes of species B are more closely related to the genes of C than they are to those of A, but 12% of the genes of B are more closely related to those of A and 12% of the genes of C are more closely related to those of A." (I am oversimplifying the matter, since species A, B and C each have many copies of any gene and the phylogeny of these copies will be considerably more complex than the three-copy phylogenies depicted in Figure 8. The important thing is that the phylogeny of all the copies will probably *not* show all copies from A, B and C forming respective monophyletic groups, and those from B and C together forming a monophyletic group. There are many possible violations of this pattern, and different genes will show different violations.) The species phylogeny no longer seems like a single, simple entity, but rather appears more like a statistical distribution. Of course, there may be some dominant pattern that appears again and again, and we might draw this pattern when we draw the "species phylogeny." But this dominant pattern is an abstract statistical concept, like the mean or mode of a population. Under this view of "species phylogeny," the tubes drawn in Figure 8 are misleading, because they imply some barrier that delimits the edge of a species phylogeny and within which the genes are forced to bounce around. If the species phylogeny concerns itself only with realized genetic connections, then the tubes indicate central tendencies only in the descriptive, statistical sense, not in the sense of constraining forces.

The reader might wonder if the need to describe frequencies of different classes of gene trees could be obviated by changing how we delimit species, for instance, using the genealogical species concept of Baum and Shaw (1995). However, the species concept does not change the facts: gene trees agree or not regardless of what we say about them. Suppose the bulk of genes agree on a history that we can usefully consider as the divergence of populations, and that we want to study this. We could study these populations regardless of whether or not they individually fail the exclusivity test of Baum and Shaw; the only difference would be whether we label the studies as intraspecific or interspecific. In addition, not all the burden of gene tree disagreement can be shifted to population biology, because exclusivity of coalescence of species does not guarantee gene tree agreement deeper in the phylogeny. For instance, two sister species that individually satisfy exclusivity might fail to form an exclusive

group together, insofar as trees of different genes can still disagree as to whether the two species are sister species and to whom they are related.

I find a view of a species phylogeny focusing only on genetic connections to be rather stark. It fails to incorporate the notions of populations and species as individual entities with forces maintaining their distinctness and that participate in higher-order processes like species selection. It views populations as just bags of genes that happen to have been entangled in their history. An argument can therefore be made that a species phylogeny should represent more than actual genetic connections, and instead should imply also that there were forces that held (or are holding) each species lineage together, and that new forces arose to split the lineages at the branch-points in the tree. (By "forces" I mean the factors or mechanisms, like geographical and reproductive isolation, that a species or its context possess that have a tendency to promote breeding within and limit interbreeding between species.) If the lines drawn to depict a phylogenetic tree are to represent species, and species are to be more than just bags of genes with common history, then somehow the phylogenetic tree must be read as "the branching pattern depicting the successive splitting of the bundles of genetic connections *and* the development of forces causing these splits." Even if the forces are not specified, they can be implicit, and the tree can depict the history of potentials: of what matings could have occurred.

This is precisely the notion of species phylogeny used in Figure 8 and the related literature. The species phylogeny of Figure 8 would tell us that there was an original panmictic population. At some point it was sundered into two panmictic populations, and then finally three. This is a probabilistic model: it tells us what matings could have occurred (random within populations), and what couldn't (none between populations), and it yields probabilities for various possible patterns of gene trees. But it doesn't tell us what actually happened in terms of gene trees. All the genes of species B *might* still be more closely related to the genes of species A than to those of C, as improbable an outcome as that might have been. The species phylogeny would therefore be a model specifying the probabilities of what could have occurred, and not an actual genetic history. The approach to testing it could be straightforward: under such a species tree, different gene trees would occur with different probabilities, and the species tree could be chosen as that which confers the highest probability on the "observed" gene trees (or, ultimately, on the observed sequence differences).

We are faced with two concepts of species phylogenies, one in which the tree outlines the genetic connections that happened and one that outlines the genetic connections that could have happened. (Other concepts are, of course, possible, especially if we retreat into operationalism, but I will not address these here.) The question is, do we want our historical account, or phylogenetic tree, to include the past forces or potentials? I do not know the answer. I suspect some systematists will be unhappy with a notion of a phylogeny that incorporates evolutionary potentials or forces. On the other hand, some systematists will also be unhappy with a notion that the species phylogeny is nothing more than a composite of gene trees. Portraying only realized genetic connections may lend greater clarity of meaning to the trees (analogous to the clarity obtained by cladistic classifications depicting branching only and not anagenesis), and may free us from commitments to certain models of process. However, process models can help organize and motivate our thinking. Of course, there is no reason we can't depict separately our hypothesis about genetic history and our models of the forces behind it.

At any rate, the disagreement of trees of different genes, which can be seen within populations, can also be seen among species. From the point of view of genetic history, there is no sharp line between population biology and phylogenetic biology.

LITERATURE CITED

Baum, D. 1992. Phylogenetic species concepts. Trends Ecol. Evol. 7: 1–2.

_____ and K. L. Shaw. 1995. Genealogical perspectives on the species problem. Pp. 289–303 *in* P. C. Hoch and A. G. Stephenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard. 53.

Cann, R. L., M. Stoneking, and A. C. Wilson. 1987. Mitochondrial DNA and human evolution. Nature 325: 31–36.

de Queiroz, K. and M. J. Donoghue. 1988. Phylogenetic systematics and the species problem. Cladistics 4: 317–338.

Donoghue, M. J. 1989. Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. Evolution 43: 1137–1156.

Felsenstein, J. 1985. Phylogenies and the comparative method. Amer. Naturalist 125: 1–15.

_____. 1992a. Estimating effective population size from samples of sequences: Inefficiency of pairwise and segregating sites as compared to phylogenetic estimates. Genet. Res. 59: 139–147.

_____. 1992b. Estimating effective population size from samples of sequences: A bootstrap Monte Carlo integration approach. Genet. Res. 60: 209–220.

Harvey, P. H. and M. D. Pagel. 1991. The Comparative Method in Evolutionary Biology. Oxford Univ. Press, Oxford.

Hein, J. 1990. Reconstructing evolution of sequences subject to recombination using parsimony. Math. Biosci. 98: 185–200.

Hudson, R. R. 1983. Testing the constant-rate neutral model with protein sequence data. Evolution 37: 203–217.

_____. 1990. Gene genealogies and the coalescent process. Oxford Surv. Evol. Biol. 7: 1–44.

_____, M. Slatkin, and W. P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132: 583–589.

Maddison, W. P. and D. R. Maddison. 1992. MacClade: Analysis of Phylogeny and Character Evolution. Version 3.0. Sinauer Associates, Sunderland, Massachusetts.

_____ and M. Slatkin. 1991. Null models for the number of evolutionary steps in a character on a phylogenetic tree. Evolution 45: 1184–1197.

Mayr, E. 1963. Animal Species and Evolution. Harvard Univ. Press, Cambridge, Massachusetts.

Nixon, K. C. and Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6: 211–223.

Pamilo, P. and M. Nei. 1988. Relationships between gene trees and species trees. Molec. Biol. Evol. 5: 568-583.

Ridley, M. 1983. The Explanation of Organic Diversity. Oxford Univ. Press, Oxford.

Simpson, G. G. 1961. Principles of Animal Taxonomy. Columbia Univ. Press, New York.

Slatkin, M. and W. P. Maddison. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics 123: 603–613.

_____ and _____ . 1990. Detecting isolation by distance using phylogenies of alleles. Genetics 126: 249–260.

Tajima, F. 1983. Evolutionary relationships of DNA sequences in finite populations. Genetics 105: 437–460.

Takahata, N. 1989. Gene genealogy in three related populations: Consistency probability between gene and population trees. Genetics 122: 957–966.

GENEALOGICAL PERSPECTIVES ON THE SPECIES PROBLEM[1]

David A. Baum[2] and Kerry L. Shaw[3]

ABSTRACT. The species category marks the boundary between systematics and population biology, hence a clear understanding of species is critical for both these fields and their intercommunication. There are several distinct entities in nature that have been subsumed under the single term "species" (e.g., genealogical units, interbreeding groups, ecological units, and morphological clusters). We focus on one species-entity of particular concern to phylogenetic systematists, the "genealogical species." We start from the assumption that genealogical species are basal taxa (taxa containing no less inclusive taxa) and that they reside at the boundary between reticulate and divergent genealogy. These two assumptions are met if we define species as basal, exclusive groups of organisms, where exclusive groups are ones whose members are all more closely related to each other than to any organisms outside the group. We explore two genealogical measures of relatedness to see how they affect the implementation of this species definition. If relatedness is measured in terms of recency of common organismic descent, then species delimitation becomes problematic. We argue that this is due to measuring relatedness in terms of organismal descent, and suggest that we should instead focus on the genealogical histories of genes. This can be accomplished with insights from coalescent theory and in so doing a tractable, theoretically consistent definition of the genealogical species can be developed.

The species is probably the most important concept in organismic biology, yet it is also one of the most problematic and confusing. Despite many attempts to clarify the definition of the term "species" the problem still attracts much attention and intense controversy. The dominant approach to the problem is to view the species as a single entity with many distinct biological properties (e.g., Mayr, 1942; Wiley, 1978; Paterson, 1985; Templeton, 1989). In this framework the "species problem" is the search for the single property or combination of properties that define **the** species. However, a number of biologists reject the assumption that only one entity is being referred to by the term "species." Instead, they emphasize that the term has been used to refer to several, equally meaningful but distinct entities (e.g., Holsinger, 1984; Kitcher, 1984; de Queiroz and Donoghue, 1988; Wilkinson, 1990; Ereshefsky, 1992). Under this pluralistic perspective the primary aim is to conceptually separate the species-entities that have been heretofore conflated, characterize each of them rigorously, and develop a terminology that avoids confusion (Ereshefsky, 1992).

We subscribe to the pluralistic conception of species and in this paper focus on a species-entity of particular concern to phylogenetic systematists, the "genealogical species." Starting from some minimal assumptions, we proceed to develop a rigorous definition of this

[1]For useful discussion and comments on earlier drafts of this paper we would like to thank: John Beatty, Joel Cracraft, Keith Crandall, Jerrold Davis, Kevin de Queiroz, Michael Donoghue, Jeff Doyle, Marc Ereshefsky, Anne Gerber, William Hahn, Richard Harrison, Robert Kowal, Melissa Luckow, Wayne Maddison, and Elliott Sober. We are grateful to Michael Donoghue for his efforts in arranging the symposium. David Baum thanks Ken Sytsma and the members of his lab for their support during the writing of this paper. Kerry Shaw thanks the Templeton lab for many useful discussions on the subject of species.

[2] Botany Department, University of Wisconsin, Madison, WI 53706, U.S.A. Current address: Harvard University Herbaria, 22 Divinity Ave., Cambridge, MA 02138, U.S.A.

[3]Biology Department, Washington University, St. Louis, MO 63130, U.S.A. Current address: Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, NY 14853-2701, U.S.A.

entity using the terminology and assumptions of phylogenetic systematics. Our starting assumptions are (1) genealogical species are basal taxa (*sensu* Cracraft, 1989), that is, taxa that contain no included taxa, and (2) genealogical species reside at the boundary between reticulate and divergent genealogy. Before defining the genealogical species we will discuss these two premises and their most salient implications.

SPECIES ARE BASAL TAXA

If species are basal taxa then whatever criteria are used to define taxa must also apply to species. So what are taxa? Within phylogenetic systematics[4], taxa are groups of organisms formally recognized because they share a common genealogical history (de Queiroz, 1992; de Queiroz and Gauthier, 1992). In defining taxa, one therefore only considers the evolutionary history of groups of organisms, ignoring both their current properties and their future fate (Lidén and Oxelman, 1989; Kluge, 1990; Lidén, 1992). While we do not deny that the current properties and future fate of organisms are important, they are irrelevant to the definition of phylogenetic taxa and the genealogical species described here. We are interested in the species as a product of evolution, not a player in evolution. Thus, while some biologically important species-entities may be defined on the basis of either current properties (e.g., interbreeding, morphological similarity) or future tendencies (e.g., persistence as an independent evolutionary lineage), these species-entities are not conceptually equivalent to the genealogical species discussed here.

In phylogenetic systematics, monophyly is considered the most important historical attribute of taxa. However, despite its importance in systematic theory, a monophyletic criterion cannot be used to define taxa if species are to be basal taxa (Nixon and Wheeler, 1990). This is true whether one uses a narrow or broad definition of the term monophyly. The narrow concept (e.g., Hennig, 1966; Nixon and Wheeler, 1990; Wheeler and Nixon, 1990) defines a monophyletic group as a group of species descended from a common ancestral species. Given the use of the term "species" in this definition, it is clear that species cannot be considered monophyletic (McKitrick and Zink, 1988). Thus, if taxa are defined as monophyletic groups in the narrow sense, species cannot be taxa.

The alternative position defines a monophyletic group more broadly as a group descended from a common ancestor, where ancestors are any entities that are capable of leaving descendants (e.g., taxa, populations, single organisms, breeding pairs, cells; de Queiroz and Donoghue, 1988). Under this definition, monophyly can logically apply to species, but species would not be basal taxa as monophyletic groups of organisms occur at all levels from higher taxa to family groups. Although monophyly, defined in the broad sense (the sense we will use for the remainder of this paper), may be a necessary property of some taxa, it is not a sufficient criterion for taxa since many monophyletic groups are not taxa. Some other historical property is therefore needed to define taxa.

We suggest that taxa should be defined as "exclusive" groups of organisms, where an exclusive group is one whose members are all more closely related to each other than to any organisms outside the group (de Queiroz and Donoghue, 1990b; Baum, 1992). The most important characteristic of an exclusive group is that although one exclusive group may be nested within another, two exclusive groups cannot overlap. This means that exclusive groups can only be delimited when relationships are hierarchic. Hence, if taxa are defined as exclusive groups of organisms they will always be hierarchically related to each other.

[4]"Phylogenetic systematics" is used here to refer to the school of systematics that stresses the primacy of evolutionary history (exemplified by de Queiroz and Gauthier, 1990). It should not be confused with "cladistics" which emphasizes the hierarchical distribution of characters (exemplified by Nelson, 1989). This distinction is discussed in more detail by de Queiroz and Donoghue (1990b).

Since we start from the premise that genealogical species are taxa, then they too are exclusive groups. Furthermore, since species are basal taxa, species cannot contain nested within them any other exclusive groups. Thus, we define genealogical species as basal, exclusive groups of organisms.

SPECIES RESIDE AT THE RETICULATE/DIVERGENT BOUNDARY

Our second starting premise is that genealogical species reside at the boundary between reticulate and divergent genealogy. It is therefore necessary to examine why we think such a genealogical discontinuity exists and what form it takes. Below we describe the two basic genealogical patterns, divergence and reticulation, by contrasting the genealogies of sexual and asexual organisms.

Asexual organisms have purely divergent genealogies. Each individual has only one parent in each previous generation, which means that all the organisms existing at any one moment in time can be arranged into a strictly hierarchical pattern of relationships. As long as reproduction is asexual, this hierarchical structure will exist across all levels of biological organization, stretching from the highest taxonomic levels to the individual organisms.

Sexual organisms have a fundamentally different genealogy due to the fact that each individual has two or more parents in all previous generations. The genealogy is "reticulate," with ancestor-descendant lineages that anastomose through interbreeding. Therefore, organisms form overlapping patterns of relationship. For example, each of us is potentially a member of two groups of first cousins, one on our father's side and one on our mother's side. However, one's maternal and paternal first cousins are not each other's first cousins. Hierarchies (as defined by Gregg, 1954) cannot contain overlapping sets and, thus, this example demonstrates that the pattern of relationships arising from a reticulate genealogy is fundamentally non-hierarchic.

In contrast to the relationships within sexually reproducing populations, the higher taxa formed by biparental organisms can be arranged in a non-overlapping nested array. The divergent genealogy that is inferred to have generated this hierarchical structure is termed a "phylogeny." Biparental organisms thus have a distinctive genealogical pattern. The organisms within a population are related by a reticulate pattern of descent whereas the higher taxa to which these organisms belong are related by a divergent pattern of descent (the phylogeny). Clearly there must be some boundary (however fuzzy) at which the reticulate genealogies of sexual organisms become transmuted into the divergent relations of higher taxa. There must be a taxon whose relationships with other taxa are predominantly divergent but whose parts (the organisms ascribable to the taxon) are related to each other by a predominantly reticulate genealogy. We refer to this entity as the "genealogical species[5]."

As discussed above, exclusive groups of organisms are hierarchically related to each other and, thus, the existence of exclusive groups indicates that a genealogy is divergent. Similarly, when genealogies are reticulate, exclusive groups cannot arise. Therefore, the lowest level at which exclusive groups emerge should approximate to the reticulate/divergent boundary and, hence, defining species as basal, exclusive groups of organisms satisfies both our starting assumptions.

[5]This concept is a phylogenetic species concept insofar as it is defined primarily on the basis of historical relatedness rather than current properties or future fate. However, it will become clear that it has several novel features that distinguish it from other phylogenetic species concepts (e.g., Cracraft, 1983, 1989; Donoghue, 1985; Mishler and Brandon, 1987; de Queiroz and Donoghue, 1988, 1990a, 1990b; Nixon and Wheeler, 1990; Wheeler and Nixon, 1990; Davis and Manos, 1991; Davis and Nixon, 1992; reviewed by McKitrick and Zink, 1988; Avise and Ball, 1990; Frost and Hillis, 1990; Baum, 1992). We use the term "genealogical species" to avoid confusion with these other concepts.

EXCLUSIVITY AND THE EVALUATION OF RELATEDNESS

Central to the definition of exclusivity is degree of relatedness, and criteria are needed whereby relatedness can be evaluated. In accordance with our emphasis on evolutionary history we discuss two genealogical measures: recency of common organismal descent and recency of genetic coalescence. The former approach focuses on the genealogy of organisms, whereas the latter focuses on the genealogies of genes. Our main claim is that, despite its simplicity, recency of common organismal descent leads to severe problems when used to define genealogical species. We show this to be the case whether one views taxa as comprising both ancestors and descendants (the "time-extended" approach) or just contemporaneous organisms (the "time-limited" approach). We argue that the problems with evaluating relatedness through organismic ancestry arise because reticulation is discernible only at the gene-level and not at the organismal level. Thus, only an approach that looks at genetic coalescence will successfully place genealogical species at the reticulate/divergent boundary.

Recency of Common Ancestry. In phylogenetic systematics, degree of relatedness is traditionally determined by recency of common descent. Organisms are considered closely related if they share a common ancestor in the recent past, distantly related if their most recent common ancestor (MRCA) is ancient. Using this measure of relatedness, an exclusive group of organisms (a taxon) is one whose MRCA existed more recently than the common ancestor of any member of the group and any organism outside the group.

One obvious advantage of this definition of exclusivity is that, in a divergent genealogy, monophyletic groups and only monophyletic groups are exclusive. As Hennig (1966) realized, monophyletic groups can be identified by the possession of synapomorphies. This means that when a genealogy is divergent, cladistic methods can identify exclusive groups of organisms (i.e., taxa). However, the symmetry of monophyly and exclusivity does not apply when genealogies are reticulate (de Queiroz and Donoghue, 1990b; Baum, 1992). In a reticulate genealogy, monophyletic groups of organisms occur (although in practice they are difficult to detect because synapomorphy ceases to be evidence of monophyly; de Queiroz and Donoghue, 1988; Nixon and Wheeler, 1990), but they will not be exclusive. This means that monophyly is irrelevant to the definition or practical recognition of genealogical species.

There are two alternative approaches to delimiting exclusive groups when recency of common descent is used to evaluate relationships, and these lead to quite different outcomes. The "time-extended" approach considers a group of organisms (living or not) exclusive if they share a more recent common ancestor with each other than with any organisms (living or not) outside the group. The "time-limited" approach judges a group of contemporaneous organisms exclusive if they share a more recent common ancestor with each other than with any contemporaneous organisms outside the group. We will discuss the time-extended and time-limited approaches in turn showing that, when considering organismic measures of common ancestry, neither of them places the genealogical species at the reticulate/divergent boundary.

The time-extended approach requires quite exceptional biological circumstances for exclusive groups to be formed. Consider the hypothetical phylogeny shown in Figure 1. An ancestral population has divided into two at time **t**, after which time the two descendant "lineages" have not interbred. Superficially this diagram implies that the two descendant "lineages" are non-overlapping and, hence, each shows exclusivity of descent. However, close scrutiny of the events at the time of the split reveals that in fact neither of the two descendant "lineages" are exclusive. For example, the MRCA of labeled individuals **A** and **B** is organism **C** living at time **t-3**. However, the common ancestor of **A** and **D** (a member of the other descendant lineage) is organism **E** living at time **t-1**. Under the "time-extended"

approach, "lineage 1" is therefore not exclusive (the same can be shown for lineage 2). No matter how long the two "lineages" persist without interbreeding, they will never become exclusive.

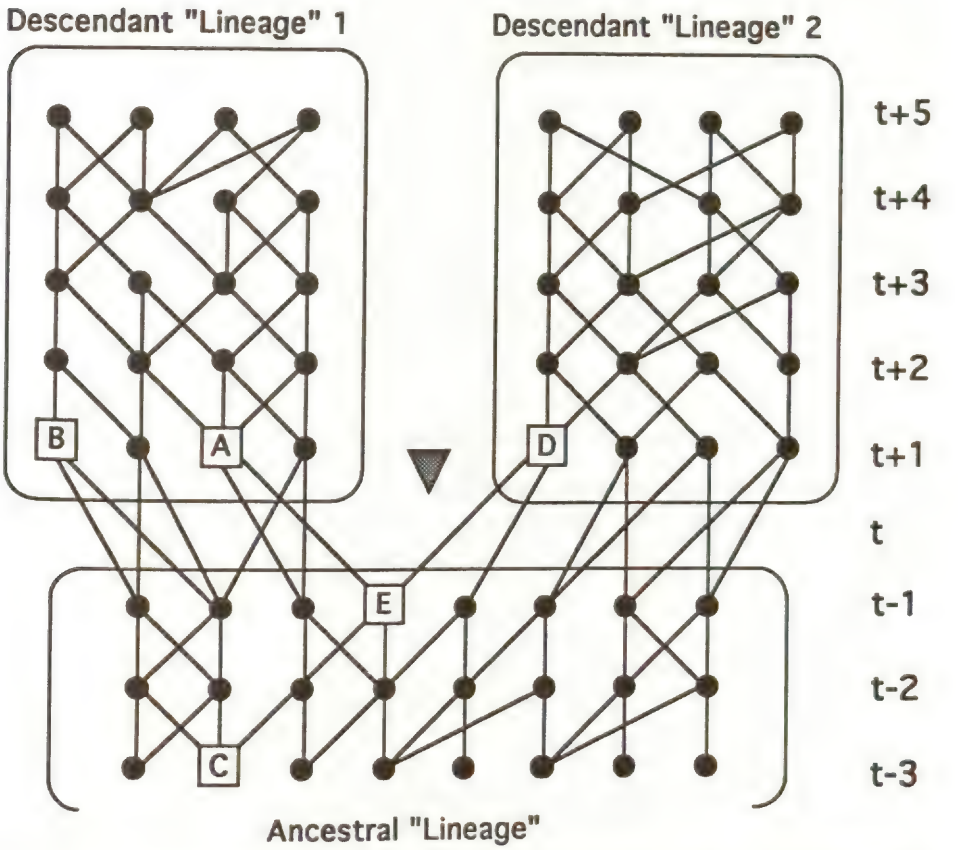


Figure 1. A hypothetical organismic genealogy of biparental, hermaphroditic annuals. An ancestral "lineage" divides at time **t** into two descendant "lineages." The MRCA of labeled organisms **A** and **B** is organism **C** and that of organisms **A** and **D** is organism **E**.

The only way that exclusive groups can form is when a single individual or breeding pair gives rise to a "lineage" whose descendants never interbreed with other "lineages." Such groups may form (e.g., when a founder population is derived from a single breeding pair) but only under very rare conditions in nature. Furthermore, evolutionary theory predicts that divergence and the formation of hierarchical structure occurs without such extreme "bottlenecks." Thus, it is clear that measuring relatedness using recency of common ancestry under a time-extended view of taxa results in genealogical species emerging well above the reticulate/divergent boundary.

Unlike the time-extended approach, the time-limited definition of exclusivity succeeds in separating the two descendant "lineages" in Figure 1. Looking at the most recent generation only (**t+5**), the four members of each hypothetical "lineage" share a more recent

ancestor with each other than with any of the organisms in the other "lineage." They are therefore exclusive and, assuming they contain within them no nested groups, they would be considered genealogical species. This is encouraging because it means that widely accepted modes of speciation (e.g., allopatric or parapatric) can generate exclusive taxa without passing through the extreme bottlenecks required under the time-extended view.

The problem with the time-limited definition of exclusivity is that exclusive taxa (species) will also emerge through chance patterns of mating within panmictic populations. Figure 2 illustrates this situation using a hypothetical reticulate genealogy of a population of hermaphroditic annuals. The organisms living in the current generation would be assigned to five exclusive groups (**A**, **B**, **C**, **D**, and **E**). For each group one MRCA is labeled, and it can be seen that this MRCA lived closer to the present than the ancestor shared with any contemporaneous organisms outside the group. Of these exclusive groups, **A**, **B**, and **D** are basal, exclusive groups in that they contain no nested exclusive groups. Thus, if exclusivity is defined using recency of common descent under the time-limited view, then **A**, **B**, and **D** would have to be considered genealogical species. However, to elevate such small and closely related groups of organisms to the rank of species clearly would be of little practical value. Further, we maintain that recognizing this level of genealogical structure results in placing the species taxon well below the reticulate/divergent boundary.

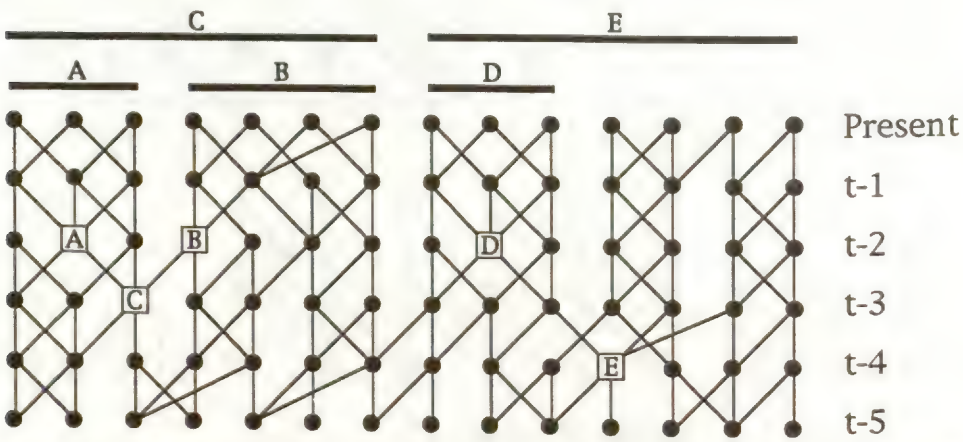


Figure 2. A hypothetical organismic genealogy for a population of biparental, hermaphroditic annuals. Time-limited exclusive groups (see text) are marked with a bar. One of the MRCAs of each exclusive group is labeled with the same letter as the group.

How can we retain the exclusivity criterion without having to recognize species at such low taxonomic levels? In other phylogenetic species concepts this problem (or analogous problems) was solved by invoking current or future interbreeding (as opposed to past interbreeding, i.e., reticulate genealogy) as a secondary criterion of species delimitation. Cracraft (1983, 1989), Nixon and Wheeler (1990), Davis and Manos (1991), and Davis and Nixon (1992) use a reproductive cohesion criterion (or some variant thereof) to individuate the units within which diagnostic character variation is indicative of a "parental pattern of ancestry and descent." Mishler and Brandon (1987) employ current interbreeding as one of several criteria that permit the assignment of monophyletic groups to the rank of species, whereas de Queiroz and Donoghue (1988) suggest that biological populations be terminal units in the search for monophyletic species.

Despite a long-standing tradition within evolutionary biology of incorporating interbreeding criteria into species concepts, there are good reasons for rejecting it here. As discussed earlier, we are interested in a species taxon that is a product of evolution and therefore this taxon must only be defined on the basis of history (Lidén and Oxelman, 1989; Kluge, 1990; Lidén, 1992). Thus incorporating information on the inferred fate of groups of organisms into a species definition would undermine our most basic assumptions about the nature of taxa.

The need for an *ad hoc* criterion (such as interbreeding) to elevate the level at which species emerge is an inevitable consequence of defining exclusivity in terms of recency of common organismic descent. We do not suggest rejecting the exclusivity criterion as it is an important and powerful concept in phylogenetic theory. Instead, the problems encountered can be overcome by retaining exclusivity but using a more appropriate measure of genealogical relatedness.

Recency of common organismic descent as a measure of relatedness focuses on a level of biological organization that does not manifest reticulation and divergence. This, we believe, is why it causes genealogical species to emerge above (time-extended view) or below (time-limited view) the reticulate/divergent boundary. An organismic measure of relatedness implicitly ignores the fact that many of the genes found in a group of organisms do not come from their MRCA. Many ancestors other than the most recent contribute to a group's genetic make-up and should be considered in the evaluation of relatedness. This mosaic pattern arises because sexual reproduction involves recombination: the reshuffling of gene combinations from generation to generation. Indeed, it is recombination, not simply biparental reproduction, that is the cause of reticulation. By focusing on organismal MRCAs, genetic reshuffling is undetectable and therefore reticulation is obscured.

These problems can be overcome if we change our focus from organismal to genic genealogy. Whereas the previous focus on organismic pedigrees conceals the mosaic history of genomes, an approach based on gene-history permits us to detect and more accurately explore the boundary between reticulation and divergence. Until recently we lacked a terminological and conceptual framework in which individual gene-histories could be divorced from the pedigrees of the organisms containing them. However, the recently developed branch of population genetics known as coalescent theory offers a framework in which individual and collective gene history can be discussed.

Exclusivity of Coalescence. Coalescent theory is an actively developing branch of population genetics concerned with tracing the genealogical history of a present-day sample of genes (see, Hudson [1990] and Maddison [1995] for useful introductions). A "gene" is defined in coalescent theory as a non-recombining genetic element (for example, the chloroplast genome could be a gene). Since genes show no recombination, by definition, gene-genealogies will always be divergent even if the genes are sampled from within a sexually reproducing population. Thus, if you take a sample of genes and trace their lineages backward through time there are successively fewer ancestral genes in previous generations. Eventually if you go far enough back in time, the genic lineages leading to the sampled genes will "coalesce" to a single lineage.

Coalescent theory suggests an alternative means to evaluate relatedness. If an homologous (orthologous) gene is sampled from two organisms one can ask: How far back in time do these genic lineages coalesce? The depth of this coalescence is a measure of relationship between the sampled genes. The further back in time coalescence occurs, the more distantly related they are. Before incorporating this measure of relatedness into our definitions of taxa and genealogical species, it is important to consider how the coalescent histories of different genes from the same set of organisms are related in both divergent and reticulate genealogies.

In a divergent organismic genealogy, such as that generated by asexual reproduction, all parts of the genome have a genealogical history that exactly matches the organismal pedigree (i.e., coalescence will occur in the MRCA). Due to the lack of recombination the whole genome can be considered a single gene (as defined above) and therefore all parts of the genome will have concordant patterns of coalescence. In contrast, the coalescent histories of genes sampled from within a sexually reproducing population will not match the organismal pedigree. Furthermore, although each gene has a divergent genealogy, different genes (alleles) within a locus and genes from different, unlinked loci may have discordant genealogies. Consequently, different genes may give conflicting estimates of the degree of relatedness between the organisms from which they are sampled. For example, a pair of siblings will be judged very closely related if at the locus considered they each received the same alleles from their parents. However, if the locus sampled is one for which they received different alleles, the siblings might be judged very distantly related.

These examples illustrate the general fact that genes will have concordant genealogical histories when the organismal genealogy is divergent, but discordant histories when organismal genealogy is reticulate. As discussed by Avise and Ball (1990), genetic concordance thus provides an objective, measurable criterion for determining whether a genealogy is reticulate or divergent. These insights allows us to redefine exclusivity using recency of coalescence: A group of organisms is exclusive if their genes coalesce more recently within the group than between any member of the group and any organisms outside the group.

To see the significance of this definition of exclusivity consider how exclusive groups (i.e., taxa) emerge. Imagine a single panmictic population. The genealogical relationships of organisms are reticulate (as in Figure 2), such that each individual organism receives a mixture of genes from its two parents. Because each gene has its own history, the genes at different loci sampled from the same pair of organisms can suggest quite different estimates of these organism's relatedness. Now imagine that an extrinsic barrier to reproduction arises which splits this population into two. Initially, a gene sampled from an organism in one descendant population is as likely to show more recent coalescence with genes in the other population as with genes in its own population. After a time, however, dependent primarily on the effective population size, some genic lineages will go extinct in one or the other (or both) descendant populations. Eventually, genes sampled from within the populations will be found to coalesce after the ancestral population split. If the two populations are isolated long enough all gene-genealogies will be concordant (as pointed out by Avise and Ball [1990]) and will indicate that the populations are exclusive. These populations then would satisfy the criteria for being basal exclusive taxa and would constitute genealogical species. However, it should be emphasized that genes at different loci will coalesce in different ancestral organisms and not all of these organisms need have been living at the same time.

Depending on the mode of inheritance and the ploidy of the genes under consideration, different genes within a genome will have different effective sizes (Templeton, 1987) and consequently different expected rates of coalescence. For example, X chromosome specific genes (in an XY or XO sex determining system) will coalesce more rapidly than autosomal genes in diploid organisms, because their effective population size is 3/4 that of autosomal genes. Likewise, genes favored under directional selection will coalesce more rapidly than neutral genes, whereas genes under frequency dependent or balancing selection will coalesce more slowly. We expect the depth of the reticulate/divergent border to depend on mean times to coalescence. However, even when a vast majority of the genome is concordant in supporting the exclusivity of a particular group of organisms we expect some genes (especially those under balancing or frequency-dependent selection) to show discordant patterns. Furthermore, even for neutral genes there will be variation in actual times to coales-

cence due to stochastic factors. Thus we predict that genealogical species will have "fuzzy" boundaries. The theoretical implications of fuzziness are discussed in more detail below.

Our definition of the genealogical species resembles the definition of subspecies proposed by Avise and Ball (1990). However, they endorsed a monistic rather than pluralistic view of the species problem, and subscribed to the Biological Species Concept, which groups organisms on the basis of reproductive compatibility (Mayr, 1942). As discussed previously, reproductive criteria are not employed in the delimitation of genealogical species. Contrary to Avise and Ball (1990), we do not assume that basal exclusive groups will always be nested within reproductively compatible groups. It seems likely that under certain demographic situations, genealogical species will contain reproductively incompatible sub-groups which are not themselves exclusive. Hence, a reproductive grouping criterion may delimit either more or less inclusive groups than the genealogical criterion presented here. Furthermore, a group delimited on the basis of reproductive compatibility could show partial overlap with a group defined on the basis of exclusivity of coalescence. How reproductive and genealogical groups compare and the degree to which they are coextensive is currently an unexplored question and an exciting subject for future research.

PRACTICAL IMPLEMENTATION

To make the coalescent approach more concrete, Figure 3 gives a hypothetical example of how genealogical species can be studied in nature. Four rapidly evolving, unlinked loci have been sampled (e.g., mitochondrial DNA, chloroplast DNA, and two independently assorting nuclear genes) and one allele has been sequenced from each of eleven individuals in two different geographical populations (**a–e** from one, **f–k** from the other). The DNA sequences are used to generate four separate gene trees, each assumed to represent the coalescent histories of one of the genes.

In order to delimit exclusive groups of organisms based on these separate gene trees it is necessary to look for points of concordance among the gene trees. This can be achieved using a strict consensus[6] of the gene trees, treating the genes from the same organism as equivalent terminal taxa (Figure 3). Resolved nodes in the consensus tree indicate genetic concordance, whereas unresolved nodes indicate discordance. In the worked example, the consensus tree suggests that organisms **f–k** are an exclusive, coalescent group. These data would thus support the hypothesis that the population comprising organisms **f–k** constitutes a genealogical species. In contrast, the data suggest that the population containing **a–e** does not constitute a genealogical species. Indeed, organisms **a–e** cannot be assigned to any species as the smallest exclusive group containing them (**a–k**) is not basal (it contains the exclusive taxon **f–k**). Such a finding would suggest that **a–e** constitutes a "metaspecies" (see Donoghue, 1985; Mishler and Brandon, 1987; de Queiroz and Donoghue, 1988, 1990a; Kluge, 1989), which we would define as: a non-exclusive assemblage of organisms that can only be assigned to a taxon that contains within it at least one genealogical species. Unlike some authors, we employ the term metaspecies to describe an ontological situation (organisms that are not members of any species) rather than an epistemological one (groups that cannot be assigned to recognized species due to a lack of evidence). Accordingly, if organisms **a–e** are members of a metaspecies then even with complete knowledge of all organismic and genic relationships, these organisms could not be assigned to any genealogical species. A more extensive discussion of metaphyly is beyond the scope of this paper. However, we argue that

[6]Further work is needed to determine what methods apart from the strict consensus might be appropriate for inferring the boundary between genetic concordance and discordance. This is a particularly acute problem when more than one allele per locus from a single organism is included in which case the strict consensus method in its traditional form cannot be applied.

some pieces of the genealogy of life will inevitably be left over when we carve out genealogical species, and that these pieces must be terminologically distinguished so as to avoid them being ascribed the properties of exclusive taxa.

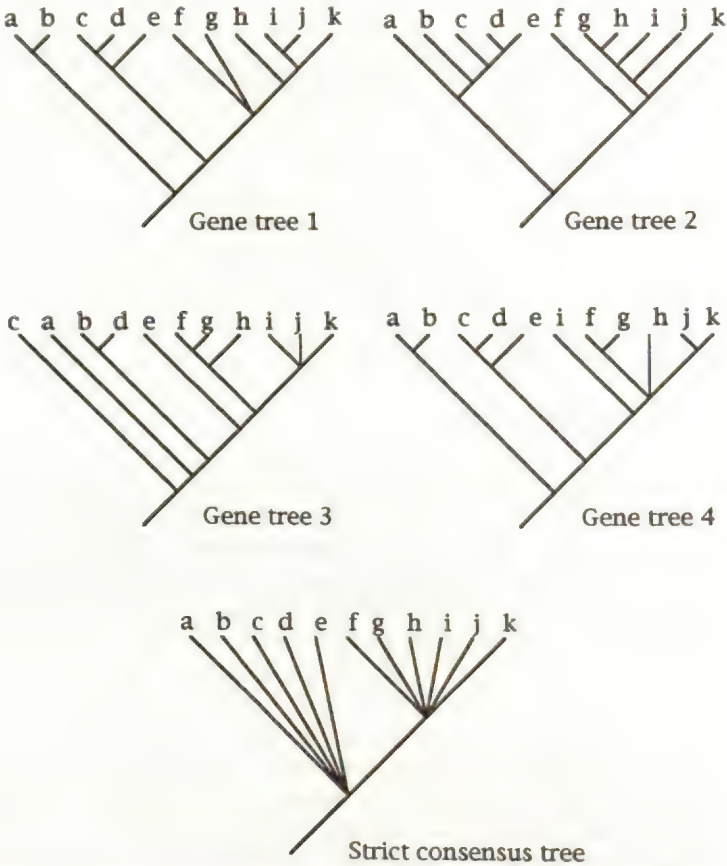

a b c d e f g h i j k
Gene tree 1
a b c d e f g h i j k
Gene tree 2
c a b d e f g h i j k
Gene tree 3
a b c d e i f g h j k
Gene tree 4
a b c d e f g h i j k
Strict consensus tree

Figure 3. A worked example of the implementation of the genealogical species concept. Eleven organisms (**a**–**k**) are sampled from two geographic populations (**a**–**e**; **f**–**k**). From each individual, four unlinked genes are sequenced and gene trees constructed using standard phylogenetic procedures. Points of genetic concordance are identified using a strict consensus of these trees (see text).

An analysis such as that described in the worked example should be seen as a first step in a process of reciprocal illumination. An increased sample of organisms or of genes may contradict a group's exclusivity. As with existing species concepts, the decision as to when a sufficiently large sample has been studied will, in practice, rest with the individual systematist and the systematic community as a whole. Likewise, the extent to which rare nonconcordant gene-genealogies (such as those under balancing selection) cause the rejection of a putative species will be determined by convention rather than any objective criteria.

While this hypothetical example illustrates how the genealogical species concept can be applied in practice, it will be clear to most systematists that the degree of study involved greatly exceeds the time and resources available for most taxonomic studies. However, we argue that this is not a reason for rejecting our genealogical perspective. As discussed by Hennig (1966), Simpson (1961), and others, the taxonomic naming of a species should be

viewed as a hypothesis. In our view, this means that the role of a species concept is not to provide a convenient recipe for "discovering" species, but rather to provide a conceptual criterion against which species-hypotheses can be judged. We consider it important to have theoretically consistent criteria for testing taxon status even if these criteria cannot always be applied due to practical limitations.

In practice, systematists are unlikely to be able to undertake full-scale coalescent analysis. However, empirical research on a few model systems could be used to determine what type of phenotypic characters are good predictors of species status. For example, the presence of fixed differences between reproductively isolated populations suggests that they have been genetically isolated long enough to allow for the fixation of unique variation. However, we emphasize that although the presence of such "diagnostic" variation can be used to hypothesize that a group of organisms constitutes a genealogical species, it cannot by itself validate the group's species status (in contrast to the "phylogenetic species concept" of Cracraft, 1983, 1989; Nixon and Wheeler, 1990; Davis and Nixon, 1992). There are many reasons why a non-exclusive group could have fixed differences from close relatives (e.g., strong directional selection on the diagnostic locus, lineage-sorting from an ancestrally polymorphic stock or the retention of a plesiomorphic character that has been superseded in close relatives). Similarly, since coalescence occurs with or without mutation, genealogical species can potentially exist without any observable diagnostic variation (though the neutral mutation rate is probably sufficiently high that genealogical species will have at least some detectable variation at the molecular level).

Another criterion that may have utility in hypothesizing that a group is a genealogical species is the presence of barriers to reproduction or different specific mate-recognition systems (*sensu* Paterson, 1985). However, the same limitations apply to these reproductive criteria as to other diagnostic variation. Reproductively isolated groups need not be distinct genealogical species (if the isolating mechanisms are under strong selection and, hence, coalesce more rapidly than the rest of the genome). Likewise, there is no reason to think that genealogical species will necessarily evolve isolating mechanisms. The difference between reproductive and non-reproductive diagnostic variation is that the former is a better predictor that the group will become exclusive in the future, even if it is not so now. This difference in future tendency is, however, irrelevant to the delimitation of genealogical species, though it is an important consideration for microevolutionary theory and for some other species concepts.

The treatment of hybridizing groups and asexual organisms are common problems for all species concepts. Hybrids cause difficulties for the genealogical species concept as well, because hybridization between species can lead to a loss of exclusivity (and hence taxon status) not just for the species involved but also for the higher level taxa in which they are nested. However, rather than sweeping hybridization under the carpet, the genealogical species concept highlights the fact that hybridization is merely a form of reticulate genealogy, and this can be analyzed from a coalescent perspective. It is therefore an empirical question whether or not genealogical species can be delimited within groups of introgressing "species" (e.g., within *Quercus* and *Helianthus*).

Asexual taxa have the same ontological status as sexual taxa in that both are exclusive groups of organisms. However in asexual organisms the absence of reticulation means they do not form genealogical species as defined here (some workers, e.g., Vrana and Wheeler [1992] have failed to recognize this important difference between sexual and asexual organisms). Although basal taxa may exist in asexual organisms (usually sib-groups) these are not comparable to genealogical species (except in so far as they are taxa). In the terminology of Donoghue (1985), asexual taxa are defined by the same "grouping criterion" as

sexual taxa (i.e., exclusivity) but lack the "ranking criteria" developed here for sexual taxa (i.e., proximity to the reticulate/divergent boundary). It may be desirable to develop a subjective ranking criterion (e.g., phenetic disjunction) to identify some asexual taxa as "species," but these would not be genealogical species.

Once recognized, genealogical species will be suitable terminal taxa for cladistic analyses. This concern has played a critical role in motivating the development of the "phylogenetic species concept" (Cracraft, 1983, 1989; Nixon and Wheeler, 1990; Wheeler and Nixon, 1990). The primary issue is that the methods of cladistics will only elucidate evolutionary history when the operational taxonomic units show non-reticulate relationships (de Queiroz and Donoghue, 1988; Nixon and Wheeler, 1990). Thus, if species are to be terminal taxa their relations must be strictly divergent. Since the genealogical species described here are related to each other by a divergent genealogy they are appropriate terminal taxa for higher-level cladistic analyses.

THEORETICAL IMPLICATIONS

Does the coalescent approach satisfy our starting premises? Our first assumption, that species are taxa, is met because all taxa including species can be viewed as exclusive groups of organisms. Our second assumption, that species reside at the boundary between reticulation and divergence, is likewise met. As our discussion has shown, only divergent genealogies generate coalescent, exclusive groups of organisms. Thus, making species basal exclusive taxa places them at the boundary between reticulation and divergence.

An important implication of the gene-genealogy approach is that exclusive groups of organisms are only meaningfully delimited among organisms living at one point in time. Thus, when relatedness is evaluated using recency of genetic coalescence, basal exclusive groups are time-limited entities (defined above). There are, nonetheless, two alternative ways of relating these basal coalescent-exclusive groups to genealogical species, differing in whether species themselves are or are not time-limited.

If genealogical species are considered to be basal coalescent-exclusive groups (as we have assumed up until now) then, clearly, they too must be viewed as time-limited entities. This approach makes species analogous to the instantaneous morphologies (semaphoronts) that make up the development pathway of organisms (Hennig, 1966). It has the advantage that species can be delimited without knowledge of the future and the only past information needed is the genealogical history that gave rise to the extant organisms. Nonetheless, viewing species (and higher taxa) as time-limited poses theoretical problems for paleontology because fossil organisms cannot meaningfully be ascribed to modern groups (see Griffiths, 1974a, 1974b). Within earlier slices of time taxa can be delimited, but in an ontological sense the taxa so delimited would not be the same as taxa existing in other slices of time (though they may comprise cross-sections of the same lineages).

If, instead of viewing genealogical species as basal exclusive groups one views them as a series of successive, basal exclusive groups connected by direct descent, genealogical species (and taxa in general) would be time-extended entities. To avoid all of life being subsumed under a single species, it is however necessary to identify criteria to mark the beginning ("birth") and end ("death") of species. Following Hennig (1966) and Ridley (1987), species can be seen as beginning at an event of cladogenesis (the point in time when a single ancestral group first breaks up into two or more descendant, basal, exclusive groups) and ending at extinction or subsequent cladogenesis. This time-extended view of species and taxa is compatible with current thinking in phylogenetic systematics (e.g., de Queiroz and Gauthier, 1990) and permits the placement of fossil organisms in extant taxa. However, it has the disadvantage that complete delimitation of species requires knowledge of genealogical rela-

tionships in previous slices of time. In addition, acceptance of a time-extended view of taxa requires major modifications to the existing taxonomic system (see Griffiths, 1974b, 1976; de Queiroz and Gauthier, 1992).

Since the ramifications of the time-extended and time-limited view for systematic theory and practice remain unclear, we do not wish to advocate strongly either one. This uncertainty does not, in our opinion, undermine the utility of the genealogical species concept. Indeed, we hope that the genealogical species concept will lead to renewed interest in temporal-extent as a factor in systematics, thereby improving our understanding of the ontological status of species and other taxa.

An implication of the genealogical species concept that conflicts with traditional systematic thinking is that species have inherently fuzzy boundaries. This fuzziness is not the result of ignorance, but is due to the different rates of coalescence of different parts of the genome (caused by a combination of stochastic and selective processes). Consequently, there is no single point in time at which the boundary between reticulation and divergence is crossed. It is important to stress that this fuzziness is not an attribute of the concept itself, which like any theory or model is an idealized abstraction, but arises in the application of the concept to real organisms. Few (if any) real objects have absolute boundaries if they are observed carefully enough. Nonetheless, provided the boundary around an entity corresponds to some (however gradual) discontinuity, a conceptual description of the entity can have great heuristic value (Simpson [1961] makes a similar argument with regard to his evolutionary species concept). For example, the biological population is an important concept in microevolutionary theory despite lacking a sharp boundary (in this case expressed as probability of gene exchange). Likewise, the fuzzy boundary of the genealogical species may clash with systematic practice but it does not threaten the concept's theoretical value in macroevolutionary theory.

The genealogical species concept provides a point of contact between the fields of population biology and systematics. In defining this entity we have employed models of gene-genealogy developed by coalescent theory, a branch of population genetics, to describe and define taxa, the basic units of the nested hierarchy studied in systematics. The approach therefore provides a conceptual link between the process of genetic isolation and the hierarchical structure of nature. This can be seen clearly by the important empirical questions that the genealogical species concept poses. Just as the biological species concept (Mayr, 1942) focused attention on reproductive isolating mechanisms, the genealogical species concept focuses attention on the mechanisms by which divergent (phylogenetic) patterns of relationship emerge out of reticulating patterns. The combined insights of coalescent theory and modern phylogenetic systematics allow the problem to be clearly stated and, we believe, permit the development of an empirical research program bridging both population genetics and systematics.

It is beyond the scope of this paper to contrast the genealogical species with the many published "solutions" to the species problem. Our opinion is that even if this genealogical species is accepted as being a meaningful entity, this would not make other species concepts obsolete. There are many criteria by which one can structure knowledge of biodiversity. Many of these criteria are important and, hence, the entities delimited are necessary theoretical concepts (a point stressed by de Queiroz and Donoghue, 1988). In the case of the term "species," several entities of biological importance reside at a similar biological level (Ereshefsky, 1992), the most obvious being genealogical units (genealogical species), interbreeding units (i.e., biological populations), phenetic units (i.e., morphological clusters), and ecological units (i.e., filled niches). Although we maintain that within phylogenetic systematics the genealogical species is the entity we should be concerned with, other species-

entities may be relevant in different theoretical contexts. It is therefore important to dismantle the multiple expectations biologists have of "species" and characterize each distinct species-entity independently of the others. We can then evaluate empirically whether these different entities are coextensive in nature. If they match entirely then we can return to a single, universal "species" concept. If, as is much more likely, their boundaries match under only a limited set of situations, we will be forced to decide which entities are relevant in which theoretical contexts and which is the species-entity that should be recognized in our systems of classification.

LITERATURE CITED

Avise, J. C. and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surv. Evol. Biol. 7: 45–67.

Baum, D. A. 1992. Phylogenetic species concepts. Trends Ecol. Evol. 7: 1–2.

Cracraft, J. 1983. Species concepts and speciation analysis. Curr. Ornith. 1: 159–187.

_____. 1989. Speciation and its ontology: The empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. Pp. 28–59 *in* D. Otte and J. A. Endler (eds.), Speciation and its Consequences. Sinauer Press, Sunderland, MA.

Davis, J. I. and P. S. Manos. 1991. Isozyme variation and species delimitation in *Puccinellia nuttalliana* complex (Poaceae): An application of the phylogenetic species concept. Syst. Bot. 16: 431–445.

_____. and K. C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Syst. Biol. 41: 421–435.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. Bryologist 88: 172–181.

Ereshefsky, M. 1992. Eliminative pluralism. Philos. Sci. 59: 671–690.

Frost, D. R. and D. M. Hillis, 1990. Species in concept and practice: Herpetological applications. Herpetologist 46: 87–104.

Gregg, J. R. 1954. The Language of Taxonomy; an Application of Symbolic Logic to the Study of Classificatory Systems. Columbia Univ. Press, New York.

Griffiths, G. C. D. 1974a. On the foundations of biological systematics. Acta Biotheor. 23: 85–131.

_____. 1974b. Some fundamental problems in biological classification. Syst. Zool. 22: 338–343.

_____. 1976. The future of Linnaean nomenclature. Syst. Zool. 25: 168–173.

Hennig, W. 1966. Phylogenetic Systematics. Univ. Illinois Press, Urbana, IL.

Holsinger, K. E. 1984. The nature of biological species. Philos. Sci. 51: 293–307.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Oxford Surv. Evol. Biol. 7: 1–44.

Kitcher, P. 1984. Species. Philos. Sci. 51: 308–333.

Kluge, A. G. 1989. Metacladistics. Cladistics 5: 291–294.

_____. 1990. Species as historical individuals. Biol. & Philos. 5: 417–431.

Lidén, M. 1992. Species—where's the problem? Taxon 41: 315–317.

_____ and B. Oxelman. 1989. Species—pattern or process? Taxon 38: 228–232.

Maddison, W. 1995. Phylogenetic histories within and among species. Pp. xx–xx *in* P. C. Hoch and A. G. Stevenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard. 53.

Mayr, E. 1942. Systematics and the Origin of Species from the Viewpoint of a Zoologist. Columbia Univ. Press, New York.

McKitrick, M. C. and R. M. Zink. 1988. Species concepts in ornithology. Condor 90: 1–4.

Mishler, B. D. and R. N. Brandon. 1987. Individuality, pluralism, and the phylogenetic species concept. Biol. & Philos. 2: 397–414.

Nelson, G. 1989. Cladistics and evolutionary models. Cladistics 5: 275–289.

Nixon, K. C. and Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6: 211–223.

Paterson, H. E. H. 1985. The recognition concept of species. Pp. 21–29 *in* E. S. Vrba (ed.), Species and Speciation. Transvaal Museum, Pretoria.

de Queiroz, K. 1992. Phylogenetic definitions and taxonomic philosophy. Biol. & Philos. 7: 295–313.

_____ and M. J. Donoghue. 1988. Phylogenetic systematics and the species problem. Cladistics 4: 317–338.

_____ and _____ . 1990a. Phylogenetic systematics and species revisited. Cladistics 6: 83–90.

_____ and _____ . 1990b. Phylogenetic systematics or Nelson's version of cladistics? Cladistics 6: 61–75.

_____ and J. Gauthier. 1990. Phylogeny as a central principle in taxonomy: Phylogenetic definitions of taxon names. Syst. Zool. 39: 307–322.

_____ and _____ . 1992. Phylogenetic taxonomy. Annual Rev. Syst. Evol. 23: 449–480.

Ridley, M. 1987. The cladistic solution to the species problem. Biol. & Philos. 4: 1–16.

Simpson, G. G. 1961. Principles of Animal Taxonomy. Columbia Univ. Press, New York.

Templeton, A. R. 1987. Genetic systems and evolutionary rates. Pp. 218–234 *in* K. S. W. Campbell and M. F. Day (eds.), Rates of Evolution. Allen and Unwin, Boston.

_____. 1989. The meaning of species and speciation: A genetic perspective. Pp. 3–27 *in* D. Otte and J. A. Endler (eds.), Speciation and its Consequences. Sinauer Press, Sunderland, MA.

Vrana, P. and W. Wheeler. 1992. Individual organisms as terminal entities: Laying the species problem to rest. Cladistics 8: 67–72.

Wheeler, Q. D. and K. C. Nixon. 1990. Another way of *looking* at the species problem: A reply to de Queiroz and Donoghue. Cladistics 6: 77–81.

Wiley, E. O. 1978. The evolutionary species concept reconsidered. Syst. Zool. 27: 17–26.

Wilkinson, M. 1990. A commentary on Ridley's cladistic solution to the species problem. Biol. & Philos. 5: 433–446.

HYBRIDIZATION AND PHYLOGENETICS[1]

Lucinda A. McDade[2]

ABSTRACT. Because of their reticulating phylogenetic histories, hybrids pose a series of problems for cladistics (= phylogenetic systematics). It has, however, been difficult to formulate these problems precisely because the term *hybrid* has been used for a wide variety of entities with simple to very complex phylogenetic histories and little to extensive evolutionary change since origination. In an effort to clarify the issues surrounding the question of how seriously hybrids will impact phylogenetics, this paper takes the simplifying approach of dealing with *primary* hybrids: those that have simple histories and have changed little since origination. The character patterns expected in such hybrids are reviewed for the character systems frequently used in systematics. The cladistic implications of each of these character patterns is explored in terms of where hybrids will be placed and with how much homoplasy and topological change. These predictions about the cladistic behavior of hybrids in turn have implications for prospects for identifying hybrids and deciding which character system(s) to use when hybrids may be present in an analysis. Combining characters for which hybrids should have different patterns of expression is a particularly powerful way to corroborate hypotheses on hybridity. This approach has been used in a number of elegant studies of hybridization; these have clarified some cases involving hybridization, but have revealed unexpected complexities including peripatetic organelles and multiple origins of allopolyploids. Taking a uniformitarian approach, it is argued that hybridization is likely to have been common over geological time and almost always between close relatives. This notion is used to examine the possible impact of derived hybrids of ancient origin on phylogenies. Finally, the evolutionary significance of hybridization is discussed, especially in light of evidence from the analysis of character patterns that suggests that hybrids may do more than simply reshuffle their parents' characters.

INTRODUCTION

Hybridization is a highly appropriate topic for a conference and subsequent volume dedicated to biosystematics. Although the term is out of fashion today, the biosystematic approach lives on quite vibrantly in my opinion. My conception of biosystematics is that its special role is to test hypotheses on species delimitations. These hypotheses are the species entities set forth in the primary literature of taxonomy (e.g., monographs, floras). The process of testing involves accumulating evidence on the biological nature of the entities referred to as species, using any of a number of techniques as sources of data (e.g., karyology, crossability studies, distribution patterns). The results of biosystematics in turn enhance the phylogenies and classifications that are the products of systematics by increasing the knowledge on which these are based. Hybridization is one of the more interesting—and troublesome—of biological natures, and has thus been a focus of much biosystematic research.

[1]My thanks to Peter Hoch for his indispensable role in organizing this conference and for the patient and gentle prodding that yielded this manuscript. Thanks also to Michael Donoghue for organizing the session that included this paper. Susanne Kaplan, John Lundberg, Elizabeth Waters, and Tom Whitham made valuable suggestions on an earlier draft of the manuscript, and Wayne Maddison graciously provided figure 1a. The research reported herein was supported in part by a grant from the U.S. National Science Foundation (DEB BSR-8507517).
[2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, U.S.A.

Because hybridization is a phenomenon that we often think of as occurring primarily near the boundary between populations and species, the topic of this paper is also appropriate for a symposium dedicated to the interface between population biology and phylogenetics. Population biologists and phylogenetic systematists have a common interest in reconstructing the histories of the entities they study. However, the nature of the historical relationships among the entities at these levels differs, with the result that the methods used by population biologists and systematists to unravel historical relationships must also differ.

Within populations of diploid, sexually reproducing organisms, individuals have reticulating patterns of relationship as a result of inter-breeding (Figure 1a). The appropriate techniques for reconstructing these reticulating histories are those that allow the unambiguous identification of individuals as parents and offspring, ancestors and descendants (e.g., Meagher, 1986). (For a view of phylogeny at the population level that is based on genes rather than on individuals, see Maddison, 1994.) In contrast, the model of evolution at and above the species level that has been accepted by evolutionary biologists since Darwin involves descent with modification and diversification by the splitting of lineages (Figure 1b). As set forth definitively by Hennig (1966), provided that taxa are monophyletic lineages, their relationships (i.e., the pattern of diversification) may be recognized from patterns of nested sets of shared derived characters (Figure 1b). Sister taxa share all but the derived conditions unique to each, whereas more distantly related groups share only those derived conditions that are common to a higher, more inclusive taxonomic group. Thus, it might be argued that whereas phylogeneticists are most interested in derived characters shared by more than one taxon (i.e., synapomorphies), population biologists are more interested in those that characterize individuals and populations (i.e., autapomorphies).

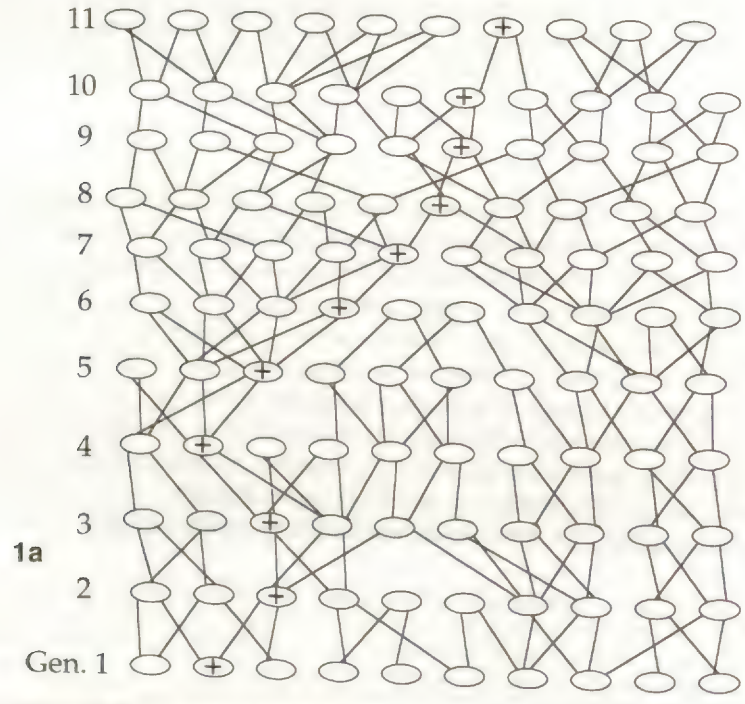


Figure 1. 1a. Schematic diagram showing reticulating relationships among individuals over 11 generations in a population. Unique characteristics or autapomorphies (marked +) of individuals permit identification of parents and their offspring.

If individuals within populations have reticulate genealogical relationships, and species and higher taxa have divergent relationships, where do hybrids come in? I think that it is most useful to define hybrids as organisms or lineages of reticulate history at a level at which divergence is expected. Using this definition, hybrids may be between individuals from isolated or differentiated populations, and there is no necessary upper limit on the taxonomic rank of the lineages to which the hybridizing individuals belong. My reasons for preferring this definition of hybridization (over those that would, for example, define hybrids in terms of taxonomic level of the hybridizing entities [e.g., Wagner, 1968]) relate directly to the models of evolution and consequently to the methods that we use in reconstructing phylogenies. When divergence is expected, most systematists have become convinced of the appropriateness of the methods of phylogenetic systematics or cladistics (i.e., using nested patterns of shared derived characters as evidence for relative recency of common ancestry [Figure 1b]). One of the problems presented by hybrids in this context is that their reticulate evolutionary history is likely to disrupt the pattern of nested sets of shared derived characters. Any disruption of these patterns is likely to prejudice our ability to sort out phylogenetic relationships in the groups with which we work. Thus, if an undetected hybrid is present, its effect may be to distort the relationships of the other taxa. A second problem that hybrids present for phylogenetics is related to the fact that cladistic methods are devised to produce branching patterns from character by taxon matrices. When a hybrid is present in a matrix analyzed cladistically, the result will not accurately portray the relationships of the hybrid. The analytical tools that we use at present cannot generate reticulating diagrams that accurately depict a hybrid's history (Figure 2a, b).

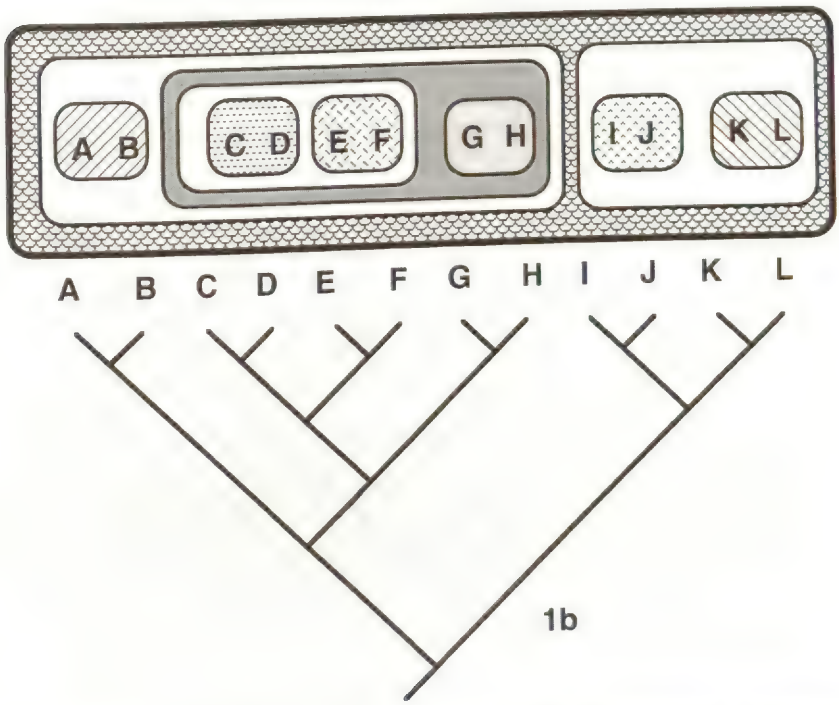
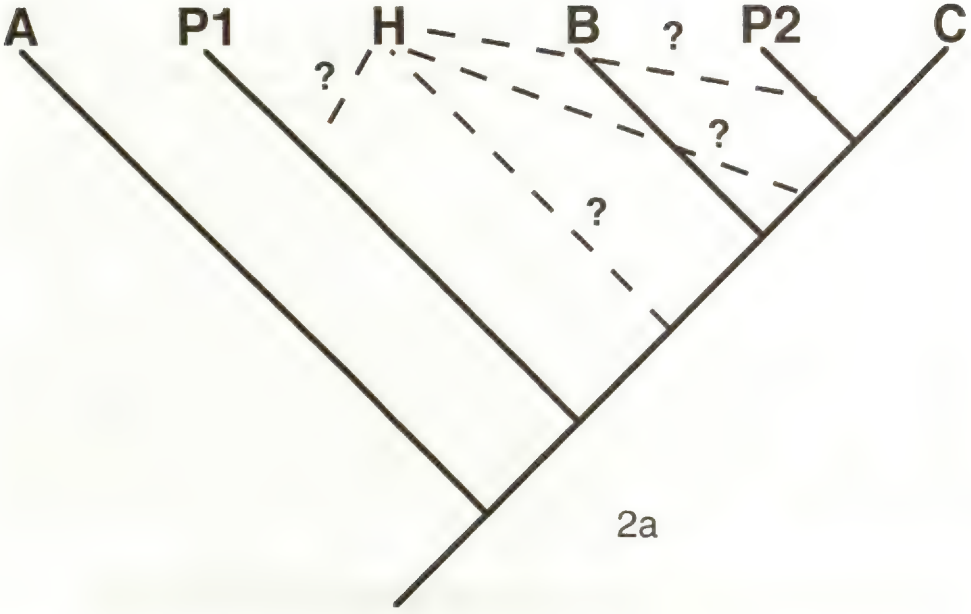


Figure 1. 1b. Divergent relationships of lineages at and above the species level; nested sets of shared derived characters (synapomorphies, above) permit recognition of relationships; after Hennig (1966).



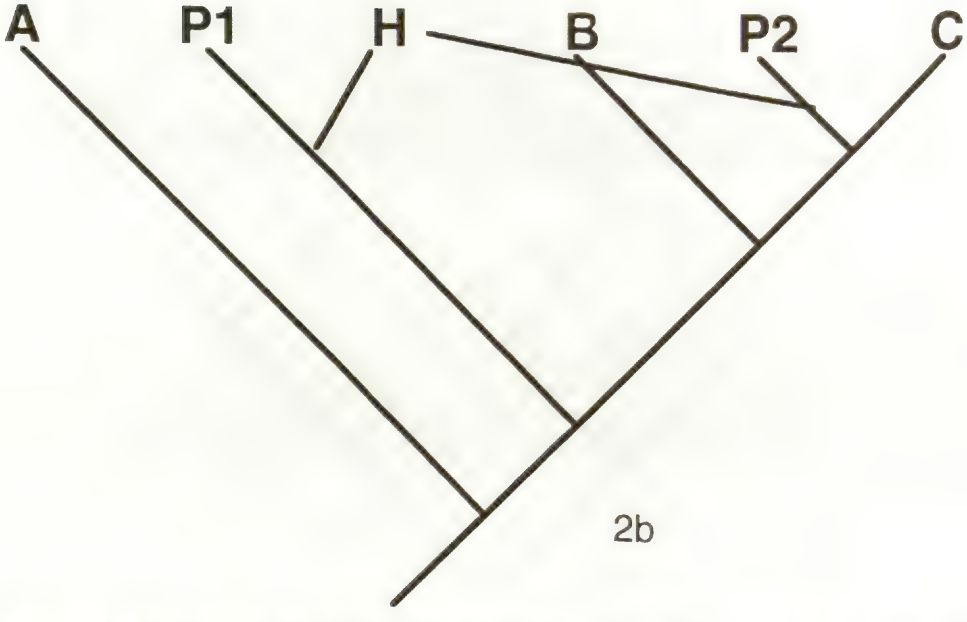


Figure 2. 2a. Cladistic analysis will place a hybrid (H) as if it were a divergently evolving taxon; its position will be determined by the most parsimonious explanation of its character states compared to other taxa in the analysis. 2b. The real phylogenetic history of a hybrid involves a reticulation between its parents, P1 and P2.

For many years, botanists have pointed to hybridization as an especially difficult problem due to the apparent commonness of hybridization among plants (e.g., Anderson, 1949; Grant, 1971; Heiser, 1949; Stebbins, 1950). In addition, plants apparently tolerate polyploidy better than many animal groups so that hybrid plants are more likely to have evolutionary futures through the restoration of fertility via polyploidy. The problem thought to face plant systematists as a result of reticulate evolution was stated especially articulately by G. L. Stebbins (1950: 365):

> The reticulate pattern of evolution which is the typical one when allopolyploidy has occurred should characterize the interrelationships between families and orders of flowering plants. If this is actually the case, an explanation might be at hand for the fact that botanists have had much more difficulty in deciding on the true interrelationships between families and orders of flowering plants than have students of insects or vertebrates....

It should be noted, however, that hybridization is reasonably common in many groups of animals, even vertebrates (e.g., mammals: Baker et al., 1989; Fedyk et al., 1991; Ferris et al., 1983; birds: Grant and Grant, 1992; Short, 1972; Tegelström and Gelter, 1990; amphibians: Arntzen and Wallis, 1991; Sage and Selander, 1979; Spolsky and Uzzell, 1984; reptiles: Densmore et al., 1989; Murphy et al., 1984; Vyas et al., 1990; fish: Ferris, 1984; Hubbs, 1955; Schultz, 1979). In fact, perhaps the best evidence for the hybrid origin of a higher taxonomic group comes from fishes: the family Catostomidae is thought to have evolved by polyploidy following a hybridization event between unknown species of cypriniforms some 50 MYA (Uyeno and Smith, 1972; Ferris, 1984).

As will have occurred to many readers, a number of the foregoing generalizations about hybrids are asserted rather than empirically supported. In fact, I would argue that a number of issues must be clarified and even reformulated as addressable questions before we can make substantial progress toward understanding the phylogenetic significance of reticulate evolution. To point to just four such issues that emerge from the above discussion:

> (1) The degree to which undetected hybrids will distort relationships among the other taxa in an analysis depends exclusively upon how the characters of hybrids are interpreted (scored) for phylogenetic analysis. How in fact do we expect the characters of hybrids to be coded relative to their parents? Are different patterns expected for the various kinds of characters used by systematists? What do these patterns imply about the cladistic behavior of hybrids?
> (2) The term hybrid has been used to refer to hybrid individuals (F_1s), hybrid populations (including any of the following: F_1s, F_2s, etc., backcrosses to either or both parents), diploid species of hybrid origin, allopolyploid species, and probably a few other biologically distinct sorts of entities as well. Areas of accord and discord will no doubt be brought into sharper focus simply by explicitly defining what is meant by the term hybrid.
> (3) What hybridizes? In the quote from Stebbins (1950) presented above, it was argued that reticulate relationships *between families and orders* were to be expected as typical of flowering plants. What does this mean in terms of the nature of the entities that are hypothesized to have hybridized?
> (4) Is it appropriate to assume that the kinds and frequency of hybridization that occur today permit estimates of what has happened in the evolutionary

past? What sorts of evidence would support (or refute) this uniformitarian approach? If we accept this idea as a reasonable point of departure, what kinds of hybridization actually are occurring today and at what frequency? Can we extrapolate from here to make predictions about what taxa of ancient hybrid origin might be like and how they will influence our efforts to unravel higher level relationships?

In this paper, I will explore the first of these issues in some detail. In so doing, I will address the second issue by suggesting a dichotomous classification of hybrids that is useful when thinking about the characters that we expect hybrids to express (and thus about their behavior in cladistics). In the final section, I will discuss the third and fourth issues, mostly by way of attempting to clarify what we need to know in order to understand how higher taxa of hybrid origin might affect phylogenetic analysis. Finally, I will discuss briefly some intriguing issues in hybridization that are emerging from recent work, and close with some thoughts on the evolutionary significance of hybrids.

KINDS OF HYBRIDS

My main goal in this paper is to explore the character patterns of hybrids compared to their parents. It therefore seems most useful to distinguish two kinds of hybrids which I shall refer to as *primary* and *derived*. *Primary* hybrids are those that are in more or less the same genetic and phenotypic condition as when they were initially formed. Hybrid individuals and populations are primary hybrids, as are allopolyploids and stabilized hybrid lineages that have evolved little. *Derived hybrids*, on the other hand, have undergone considerable evolutionary change since they originated via character evolution (i.e., the development of autapomorphies or derived characters unique to them) and/or divergence (i.e., speciation). Perhaps not happily accommodated by this dichotomy are hybrid "swarms" or populations composed of individuals representing backcrosses to either or both parents, as well as F_2s, F_3s, etc. My guess is that most such cases will present such a rich diversity of phenotypes and genotypes that they will be analyzed as problems in population biology rather than as entities suitable for phylogenetic analysis.

Hybrids have often been referred to as recent versus ancient. However, it is very rare that we actually know the ages of hybrid lineages and, in any case, the practicality of dealing with hybrids in systematics depends more on amount of evolutionary change than on age per se. Further, there is no a priori reason to reject the idea that very old hybrid lineages may exist that have not undergone much evolutionary change or that young hybrid lineages that have evolved quickly may be found. In a recent literature survey, Rieseberg and Ellstrand (1993) group hybrids as *first generation*, *later generation* and *hybrid species*. Their first generation hybrids would certainly represent primary hybrids, whereas hybrid species may be either primary or derived depending upon how much evolution has occurred since the species originated. These authors' middle group of later generation hybrids would include the complex populations referred to above.

My main focus in this paper will be on primary hybrids. I believe that there is little hope of making progress in understanding hybrids with more complex evolutionary histories if we cannot understand the characters and likely cladistic behavior of primary hybrids. Once we have clarified how primary hybrids are expected to look and behave in phylogenies, we can make informed guesses about derived hybrids, based on the kinds of evolutionary change they may have undergone.

CHARACTER PATTERNS IN HYBRIDS AND PARENTS

Table 1 is a matrix that lists the sorts of characters used by systematists (these are discussed below) and the phylogenetically informative patterns of character expression in hybrids compared to their parents. Hybrids may express the derived states (apomorphies) of both parents, the apomorphies of one parent, partially derived conditions that are intermediate between the parents, or states that are more extreme than either (autapomorphies). (Note that the expression of relatively primitive parental character states, although possible, is not phylogenetically informative and is not included in Table 1.) As long as the hybrid's characters are interpretable in comparison with its parents, this exhausts the phylogenetically informative patterns that are possible. In cases where a hybrid's characters are not interpretable compared to the parents, the hybrid condition is likely to be described as a distinct character. This should have the same cladistic impact as the "hybrid autapomorphic" category (see below).

Table 1. Matrix of phylogenetically informative patterns of character states in hybrids compared to those of their parental taxa, for character systems frequently used by systematists. Entries indicate patterns of character expression expected for hybrids for each character system. Key: ++ = hybrids should commonly be scored in this way; + = hybrids will be scored in this way under certain circumstances; (+?) = possible but unlikely that hybrids will be scored in this way. See text for full explanation.

	Character Patterns of Hybrids Relative to Parents			
Character Systems	Apomorphies of Both Parents	Intermediate	Apomorphies of One Parent	Autapomorphies
DNA: Nuclear	++			
Isozymes				
Presence/Absence	++			++
Frequency		+		
DNA: Organelles	+		++	
Chromosomes	++			
Chemistry	++	(+?)	(+?)	++
Morphology	(+?)	++	+	+

KINDS OF CHARACTERS

In the paragraphs that follow, the principal kinds of character systems used by systematists will be reviewed with the goal of determining how a hybrid is likely to be scored for such characters compared to its parents. Whenever possible, examples from the literature will be given to illustrate these points. In general, the examples come from cases where hybrid ancestry is known or strongly suspected a priori. These results thus confirm expectations for hybrid characters based on theoretical considerations, and permit generalizations to more complex cases. Note that this is not intended as a thorough treatment of character analysis, for which the reader is referred to recent treatises on phylogenetics (e.g., Swofford and Olsen, 1990; Wiley, 1981; Wiley et al., 1991). It should be noted also that Stace (1975: 58–73) provided a similar and very useful review, except that character patterns of hybrids were not placed in an explicit phylogenetic context (Stace also, of course, did not include character systems that were not in use in the mid-1970s).

DNA: Nuclear. Hybrids, by definition, combine the nuclear DNA of both parents. Thus, if each parent is monomorphic for the particular characters used (e.g., restriction sites and length polymorphisms, sequence data), their hybrid should have the patterns characteris-

tic of both. When the parents (or parental lineages) are polymorphic for a particular gene, their hybrid should have a subset of the molecular characters of each parent. In either case, for nuclear DNA characters, primary hybrids should, at least in theory, have the apomorphies of both parents.

This indeed seems to be the case for *rflp* data (restriction fragment length polymorphisms). Arnold et al. (1990) demonstrated, for example, that populations of putatively hybrid irises combined the (nuclear-encoded) ribosomal DNA types of both putative parents. Similarly, Rieseberg and coworkers' (Rieseberg, 1990; Rieseberg et al., 1990a, 1990b) elegant studies of *Helianthus* have documented a number of instances in which putative hybrids combine the restriction site and length variations characteristic of their parents. On the other hand, results may not be as clear in the case of sequence data from nuclear DNA amplified by the polymerase chain reaction (PCR). Given the dynamics of this amplification process, an initially polymorphic sample is likely to amplify unequally, reducing the likelihood that both parental DNAs would actually be sequenced. Even if both parental sequences amplify in good quantity, the resultant polymorphism is likely to be difficult to interpret (Hillis et al., 1990; Gyllenstein, 1992). These problems can be remedied by amplifying DNA multiple times and by cloning the amplified products (Gyllenstein, 1992).

An additional complication is the phenomenon of concerted evolution whereby initially polymorphic nuclear genes are gradually converted to a single version of the gene (Hillis et al., 1991). In a hybrid lineage, this process will have the effect of silencing the contribution of one parent's genome, leaving evidence of the contribution from only one parent. This phenomenon is probably more of a problem with derived hybrids (see below), but it is not yet clear how quickly concerted evolution may happen.

Isozymes. There are two ways that isozyme data are commonly used in systematics: presence vs. absence of specific alleles at a given locus and allelic frequencies. In the first case, hybrids would be expected to have at least some of the alleles present in both parents, i.e., to combine the apomorphies of their parents. If the parental species have multiple alleles for a given locus, their hybrid should have a subset of the alleles present in each parent. Thus, if for a given locus parent P1 has alleles A1, A2 and parent P2 has alleles A3, A4, their hybrid would have one of either A1 or A2 and one of either A3 or A4. In instances where numerous individuals from each parental lineage are involved in hybridization, all of the parental alleles may be represented among hybrid individuals. If the parents are fixed for a single allele at a particular locus, all of the parental alleles will be present in the hybrid. Sites et al. (1990) demonstrated, for example, that a race of unisexual, parthenogenetic lizards has most of the enzyme alleles that are characteristic of both of the bisexual races that were identified as putative parents. The results at three enzyme loci were not as expected, however, and these authors provide an insightful discussion of possible explanations (mutation and/or recombination in the hybrid, evolution in the progenitor lineages). Werth (1989) provides an excellent overview of the use of isozyme data to study cases of hybrid ancestry, with an emphasis on ferns.

As for allelic frequencies, when parental populations differ significantly in the frequency of a specific allele, a hybrid population derived from them should be intermediate in frequency (e.g., Hagen, 1990; Ness et al., 1990), although extensive samples of all three groups may be necessary to demonstrate intermediacy. As fewer individuals are involved in hybridization such that a hybrid lineage has two or a few progenitors, allele frequencies in the hybrid lineage will be the result of the same sort of founder effects that influence the genotypes of all populations derived from a few progenitors. In the extreme, one of the alleles from each of the (variable) parental lineages could be fixed in the hybrid lineage, such that

the allele frequencies would be more skewed (autapomorphic) in the hybrid than in either parent. In fact, empirical data from hybrid populations indicate that it is quite common for hybrids to have a high incidence of rare alleles compared to parental lineages. Barton and Hewitt (1985) report that, of 23 thorough electrophoretic studies of hybrids and their parental lineages, the hybrids had high frequencies of rare alleles in 19 cases. In such cases, hybrids would be scored as autapomorphic.

DNA: Organelles. The pattern of character states (nucleotide sequences or restriction sites) of mitochondria and chloroplasts (and other cytoplasmically inherited structures) in hybrids will depend upon the cytoplasmic inheritance pattern in the group in question. In most higher organisms, the pattern of cytoplasmic inheritance is maternal, but in a few groups the pattern is paternal or biparental (Birky, 1983; Corriveau and Coleman, 1988; Neale and Sederoff, 1988; Sears, 1980). Thus, in most cases, hybrid individuals should express the organelle characters of one parent or the other, combining them in cases of biparental inheritance.

The nature of the organelles present in a hybrid individual, population or lineage, also depends on the direction of the hybridization event(s) that gave rise to it. When hybridization is bidirectional, hybrid populations should have both parental types regardless of the inheritance pattern of the organelle in question. Among taxa with uniparental inheritance of organelles, in cases where the hybridization event is unidirectional (i.e., males of one parental lineage × females of the second), hybrids should uniformly possess the organelle DNA of the parental lineage that is the source of cytoplasm. For example, Rieseberg et al. (1990b) showed that a putative hybrid, *Helianthus paradoxus*, shared the enzyme alleles and nuclear DNA *rflp*s of both putative parents and had the chloroplast DNA of *H. annuus*. They thus inferred that *H. paradoxus* was indeed of hybrid origin and that *H. annuus* was its maternal parent.

Our understanding of hybridization can be enhanced by studies of reproductive biology. In animal groups where the behavior of potential mates is key to fertilization, the nature of the courtship process may be key to the direction of hybridization. Such factors may explain the introgression of coyote mtDNA into wolf populations, whereas the reverse does not seem to occur (Lehman et al., 1991). Among animals that release gametes into the environment without close proximity between mates, hybridization should be equally probable in both directions. In general, hybridization in plants should fit this latter model. However, factors such as dichogamy (the temporal separation of staminate and ovulate phases) and the behavior of particular pollinators may increase the probability of hybridization in one direction or the other. As yet, these more subtle factors that may influence character patterns in a hybrid lineage have received limited attention (but see DePamphilis and Wyatt, 1989; Sullivan et al., 1991).

Chromosomes. Morphological distinction between the chromosomes of the parental groups is one of the classical techniques for recognizing hybrids and their parents (e.g., Haufler and Zongren, 1991; Jackson, 1984; Kim and Carr, 1990; Wagner, 1971). Even when the parental chromosomes are not distinguishable by "gross" morphology, a variety of banding techniques may still permit the identification of the chromosomal contributions of each parent (e.g., Fukuda, 1984; Pipkin, 1959). Patterns of chromosome characters in hybrids compared to their parents should largely be the same as for presence/absence of enzyme alleles (which of course makes sense given the structural connection between the two character systems). Thus hybrids should combine the characteristic chromosomes (and bands) of both parents, and be scored as possessing the apomorphies of both parents.

Secondary Chemistry. The last two kinds of characters in Table 1 are perhaps the most interesting because they are the most complex. Both secondary chemistry and morphology differ from the kinds of characters explored above because they have development. The process of development involves the interaction of parental genomes to produce a phenotype in the hybrid. This process is complex and is affected by many genetic and environmental variables; as a result, a greater range of outcomes can be expected. For example, dominance interactions between alleles at the one or more loci involved in a given character may influence the phenotype of the hybrid in unexpected ways.

With respect to secondary chemistry, it is logical at first glance that hybrids would have the chemical signatures of both parents, i.e., that complementarity would occur. Indeed this has been found in a number of cases (e.g., Alston and Turner, 1963; Averett et al., 1987; Warwick et al., 1989). However, hybrids also commonly have chemical compounds that are not present in either parent (e.g., Freeman et al., 1991; Jay et al., 1989; Levy and Levin, 1975; Ornduff et al., 1973; Wyatt and Hunt, 1991). Rieseberg and Ellstrand (1993) surveyed literature reports of hybrid versus parental chemistry and found that 46% of recent hybrids had at least some novel compounds. Fahselt and Ownbey (1968) and Mears (1979) proposed models to explain this outcome. The former workers suggested that if parental lineages had each lost one step of a two-step ancestral biosynthetic pathway such that each produced a distinct compound, the entire pathway would be reconstituted in a hybrid with the result that the ancestral compound would be produced by the hybrid. Mears's (1979) models are more detailed, postulating, for example, that one parent's biosynthetic pathway might add an amino acid or a sugar residue to a compound otherwise contributed by the other parent to yield a distinctive hybrid compound.

The other two character patterns in Table 1 are also possible in hybrids and suggest specific explanations. If one parent's biosynthetic pathway is genetically dominant over the other parent's, then hybrids should have the compound of the first parent. This result might also obtain if one parent's biosynthetic pathway is much more efficient than the other's such that it uses all of the available substrate. It also seems possible that the parents' biosynthetic pathways could interact in the hybrid such that an intermediate compound is synthesized. However, one would have to know a great deal about the biosynthetic pathways and compounds involved in order to recognize a hybrid compound as intermediate (see Seaman and Funk, 1983). My guess is that most such cases would be treated as the production of unique compounds by the hybrid (i.e., hybrid autapomorphic, Table 1).

Morphology. As in the case of chemistry, a hybrid's morphological characters are the product of the developmental interaction of each parent's genetic contribution to that hybrid. (In fact, the process of development is itself of interest in hybrids compared to their parents, but has received only limited study [e.g., Burton, 1990; Leary et al., 1985; Wagner, 1962]). The accumulated wisdom from decades of working with hybrid plants is that they are morphologically intermediate between their parents (e.g., Cannon, 1909; DePamphilis and Wyatt, 1989; Kim and Carr, 1990; Ness et al., 1990; Wagner, 1968; Warwick et al., 1989). I have confirmed this pattern in a cladistic framework using hybrids between species of the genus *Aphelandra* (Acanthaceae) (McDade, 1990, 1992). It is also common that hybrids (especially polyploids) express morphological characters that are more extreme than either parent (Lewis, 1979; Miller and Behnke, 1985). Rieseberg and Ellstrand's (1993) literature survey of morphological characters in hybrids indicates that about 10% of the characters of recent hybrids are extreme compared to their parents. These authors provide a very useful discussion of the possible genetic and developmental bases of novel character expression in hybrids.

At least two other character patterns are also possible for morphological characters. In cases where one parent's gene(s) for a given character are dominant over the other's, their hybrid should express the character state of the dominant parent. Finally, hybrids may express both parental conditions. I have argued elsewhere (McDade, 1990) that the presence of both parental character states in the hybrid indicates that the parental conditions are not homologous and that they should be treated as distinct characters (see also Patterson, 1982; Spring and Schilling, 1989). Such instances are perhaps most likely to be treated as involving different characters in the absence of information about the hybrid nature of a particular taxon.

CLADISTIC IMPLICATIONS OF CHARACTER PATTERNS

There are several reasons to be concerned about the cladistic implications of each of the patterns of character expression presented in Table 1. It is, of course, the character patterns that will directly determine where a hybrid is placed cladistically and with how much "damage" in terms of added homoplasy and disruption of relationships among the other taxa in the analysis. After working through the implications for each character pattern in these terms, I will return to ask whether the placement of hybrids by cladistics is sufficiently distinctive for any of these character types to facilitate the identification of hybrids and/or their parents. In the following paragraphs, it is assumed that the given character pattern under discussion dominates the data set such that it controls the cladistic results. Other character patterns may still contribute significantly to details of the results and these will be described when noteworthy.

Hybrid combines apomorphies of both parents. Earlier workers have suggested that when a hybrid has the derived characters of both parents, it will be placed as the sister taxon to each parent in equally parsimonious trees (e.g., Hill and Crane, 1982; Humphries, 1983; Nelson and Platnick, 1980). However, this is true only when the hybrid has the same number of derived characters in common with each parent. Given the unequal branch lengths observed in most phylogenies, this is likely to occur only rarely. Instead, a hybrid that has the derived conditions of both parents should be placed proximate to the parent that has the most derived characters simply because the majority of the character changes occur along the segments of the tree leading to that parent (P2, Figure 3a). The derived character states shared with the other parent (P1) will map as homoplasies (i.e., losses or independent gains depending upon how the characters are optimized on the tree). The amount of disruption to cladistic relationships depends upon the relative stability (in terms of character support) of the nodes between the two parents. If the apomorphies that the hybrid shares with each of its parents are numerous in comparison with the number of character transitions on the intervening nodes (i.e., those starred in Figure 3a), then the hybrid's presence will pull the parental species into closer proximity (note that this is a special case of the long branch attraction phenomenon described by Felsenstein [1978]). The result may well be a cladogram that is substantially different from the correct one (Figure 3b).

Hybrid intermediate between parents. A hybrid with intermediate conditions should be placed near the base of the lineage that includes the most derived parent (P2, Figure 3c). The characters for which the hybrid has intermediate states will be fitted to the tree with no homoplasy with the result that there should be little overall increase in homoplasy. This sort of behavior by hybrids should result in little disruption of parental relationships. The results from my work with hybrids of *Aphelandra* using morphological characters confirm this idea: the majority of hybrids were placed in intermediate positions between their parents (McDade, 1992).

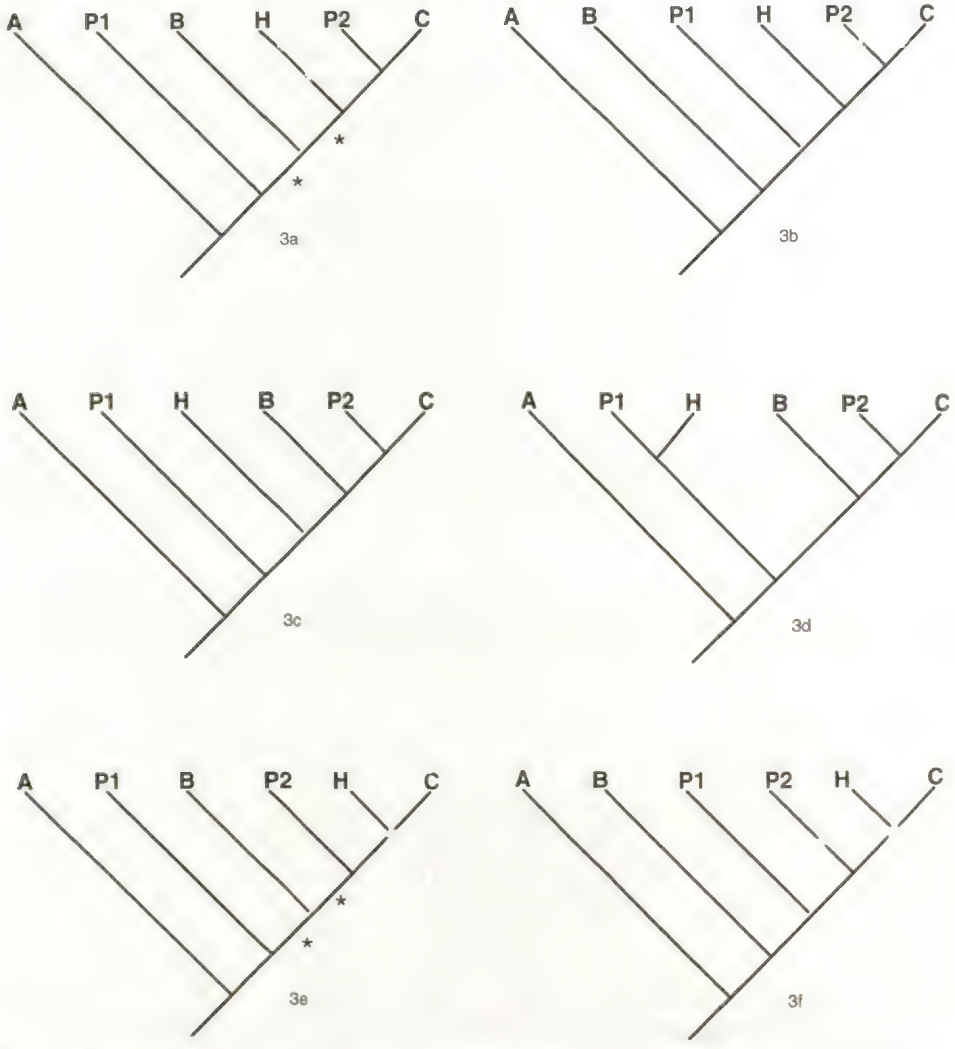


Figure 3. Cladistic placement of a hybrid (H) and its parents (P1 and P2), as a result of specific patterns of character states in hybrids. A, B, and C are taxa that belong to the group but that do not hybridize. Correct relationships among non-hybrid taxa are: (A+(P1+(B+(P2+C)))). See text for full explanation. 3a–b. Hybrid shares the derived character states of both of its parents. (3a) The hybrid is placed with its most derived parent, P2, with which it shares more apomorphies than with P1. (3b) When the stems marked with an asterisk (*) in Figure 3a are short (i.e., have few character transitions) compared to the branch lengths of P1 and P2, these latter long branches will attract and P1 will move closer to P2 as a result of the hybrid's presence. (3c) Hybrid's character states are intermediate between those of its parents. Hybrid is placed as the basal member of the lineage that includes its most derived parent, P2. (3d) Hybrid that shares the derived conditions of one parent (P1 in this example) will be placed as the sister taxon to that parent. 3e–f. Hybrid is more apomorphic than either of its parents. (3e) The hybrid is placed in a position that is cladistically distal to the more apomorphic parent (P2 in this case). (3f) When the stems between the parents (i.e., those marked with an asterisk in Figure 3e) are short compared to the branch lengths of P1, P2, and H, the presence of the hybrid will have the effect of pulling the parental species closer together cladistically.

Hybrid has apomorphies of one parent. This is perhaps the simplest of these cases; it seems clear that a hybrid that has the derived conditions of one parent would be placed proximate to that parent in cladograms with little added homoplasy and with little disruption of relationships among the other taxa (Figure 3d).

Hybrid autapomorphic. This pattern of character states should result in placement of a hybrid in a position that is cladistically distal to its most derived parent (P2, Figure 3e). The amount of homoplasy involved, as well as the degree of disruption to the relationships of the other taxa, will depend upon the hybrid's other characters. If it should share a number of derived conditions with P1 alone, these will be homoplasious. If such characters are a strong minority in the overall data set such that the character states shared by P1 and H are numerous compared to the transitions that occur along the starred segments in Figure 3e, then P1 should be pulled into closer proximity to P2 and the hybrid (Figure 3f), just as in the case of hybrids that share the derived conditions of both parents (see above and Figure 3b).

CHARACTERS, CLADISTICS, AND THE ELUSIVE HYBRID

Identification of Hybrids. Given the various problems related to the presence of undetected hybrids in a cladistic analysis, it may well be best to follow Herb Wagner's (1980) advice and remove such taxa before constructing a cladogram. However, the identification of taxa of hybrid origin is not always straightforward and one suspects that the more cryptic the hybrid, the more insidious might be its effects on efforts to unravel phylogenetic relationships. The idea that cladistics might actually help to identify hybrids therefore merits examination. If the cladistic behavior of hybrids distinguished them unambiguously from non-hybrid taxa, then they could be identified in a trial run and removed for the final analysis.

Unfortunately, from the above consideration of the implications of the character patterns of hybrids for their phylogenetic placement, it seems unlikely that hybrids will be reliably flagged by cladistics. When placed in intermediate positions between their parents, hybrids will behave much like any other cladistically intermediate taxon. It is difficult to argue that hybrid intermediacy will appear substantively different from "cladistic intermediacy" and, in the absence of an hypothesis on hybridity (see below), this character pattern alone does not seem helpful in recognizing hybrids. Similarly, when hybrids have the derived conditions of one parent and are placed as the sister taxon to that parent, it is difficult to argue from this evidence alone that the parent-hybrid pair could be distinguished from any other sister taxon pair. It is also unlikely that a hybrid that has more extreme conditions than its parents would be construed as other than a highly apomorphic taxon, of which many groups have at least one.

Characters for which hybrids combine the derived conditions of both parents, on the other hand, offer some possibility for hybrid detection, but only in an ad hoc manner. If characters are mapped onto the cladogram, the hybrid may stand out as a taxon that combines the apomorphies of two distinct taxa. Removing the hybrid from the analysis and then replacing it as a reticulation between those two taxa should significantly reduce the homoplasy required to map characters on the tree. This approach was used by Funk (1985) with considerable success. However, if a hybrid's presence has disrupted the cladogram to such an extent that its parents are brought into close cladistic proximity (see Figure 3b), then the derived characters that the hybrid shares will not necessarily map as homoplasies and might go undetected. Further, there is at least one other ad hoc explanation for this distribution pattern of character states among taxa. Near a speciation event, the daughter lineages will each have a subset of the character states present in the ancestral species. Thus, it could be argued that a putative hybrid (combining the derived conditions of its parents) is, instead, the polymorphic ancestor

that has passed a different subset of its character states to each daughter species. This amounts to suggesting that, in hypothesizing hybridity, we have the problem phylogenetically upside down.

In sum, when hybrids are intermediate, apomorphic or like one parent or the other, they are likely to "masquerade" in cladograms as perfectly normal intermediate, apomorphic or sister taxa. Only when a hybrid shares the derived states of both parents is there likely to be sufficient disruption of the nested pattern of derived conditions to suggest an hypothesis of hybridity from inspection of the cladistic results. Even in this case, however, if the presence of the hybrid causes sufficient "attraction" of the parents' branches, the result may be an apomorphic threesome made up of the two parents and the hybrid.

Corroborating hypotheses of hybridity. If, on the other hand, one begins with the hypothesis that a taxon is of hybrid origin, can cladistic analysis using one or more of these kinds of characters provide corroborating evidence? It should be clear from the above section that no one character system will suffice since other explanations (intermediacy, apomorphism, etc.) exist that are intrinsically no less parsimonious than hybridity. However, by using a combination of different character systems, one can amass data that are mutually consistent only with an hypothesis of hybridity. In practice, an hypothesis of hybridity often begins with the observation of morphological intermediacy. One might also be led to suggest that hybridization is involved when a cladistic analysis based on organelle characters places a particular taxon in a position that makes little sense given what is known of its other characters (e.g., placing a taxon whose morphological characters are intermediate within the group as sister to a highly derived taxon). Obviously, a taxon that is truly intermediate should be congruently so for all of these character systems. In contrast, one that is intermediate due to hybridity should be morphologically intermediate while having the organelle DNA of its maternal (usually) parent and combining the derived characters of the nuclear genomes of both parents. Thus compiling evidence from multiple character sources corroborates hypotheses on hybridity and makes other explanations increasingly unlikely.

A number of recent studies have taken this multiple character system approach to systematic problems where hybridity is suspected, often with quite elegant results (Doebley, 1990; Dorado et al., 1992; Doyle et al., 1990; Gastony and Yatskievych, 1992; Lavin and Doyle, 1991; Rajora, 1990; Rieseberg et al., 1990a; Soltis and Soltis, 1989; Talbert et al., 1990). A useful summary of a number of such cases is provided by Rieseberg and Soltis (1991). In many instances, these researchers are following in the intellectual footsteps of earlier biosystematists who identified problems and made as much progress as techniques available to them would allow. With a new set of powerful and relatively easily applied tools (i.e., the rapidly evolving techniques of molecular systematics), biosystematics is in a stage of rapid advancement.

Advances in Analytical Methods. Whether one seeks to identify unknown hybrids or to confirm existing hypotheses on hybridization, analytical tools that are efficient and objective would be welcome. Modifying cladistic methods to address hybridization requires allowing reticulation as a possible outcome and must be built on the patterns of character expression that are expected in hybrids compared to their parents. The first requirement has the effect of increasing very dramatically the number of acceptable topologies, even when only simple hybrid histories are permitted. The second requirement means that different algorithms will be necessary to deal with different kinds of data. At least three efforts are under way that show considerable promise of improving our ability to analyze hybrids. Hein (1990, 1993) has developed a computer algorithm that handles DNA sequences in which recombina-

tion has occurred. Basically, the program seeks to explain sequence divergence by substitutions or by recombination, assigning different weights to these phenomena. When a relatively long portion of one sequence matches that of another lineage, one recombination event is judged more likely than a series of independent substitutions. Recombination is simply reticulation at the gene level, whereas hybridization is reticulation at the organismal level. To see the potential use of this algorithm for problems involving hybridization, consider that homoplasy can be analogized to base substitutions and hybridization to recombination. To my knowledge, no attempt has yet been made to use this algorithm in cases involving hybridization.

J. D. Morefield (in Rieseberg and Ellstrand, 1993; Rieseberg and Morefield, 1994) has developed a computer program, RETICLAD, that seeks to identify hybrids based on the expectation that they will combine the characters of their parents. This algorithm should be applicable to data from character systems for which this is the expected pattern (i.e., nuclear DNA, chromosomes, chemistry and, in some cases, morphology, see above and Table 1). RETICLAD basically represents a quantification of Funk's (1985) approach to recognizing hybrids. Rieseberg and Ellstrand (1993) provide a number of examples in which the program seems to work well.

Susan Wethington at the University of Arizona (pers. comm.) is devising a program that will seek hybrids based on the expectation that they will be intermediate compared to their parents for quantitative characters and share parental states of qualitative characters in equal proportion (i.e., ca. 50% from each parent). This algorithm should be applicable to data for which this is the expected pattern (i.e., primarily morphology, also allele frequency data in some cases). This program is just beginning to be tested on real data sets, but has performed well on morphological character data for species and hybrids of *Aphelandra* (McDade, 1990, 1992).

In sum, progress is being made toward developing analytical tools that will speed and quantify our efforts to handle hybrids cladistically. To the extent that real hybrids do not have the character patterns that these programs seek, however, they will not be accurately identified. This will certainly affect the analysis of derived hybrids (see below). False positives are also possible because, as noted above, any one of the character patterns expected for hybrids can also occur in divergently evolving taxa. Still, these efforts represent important steps toward the objective analysis of hybrids.

Choice of Character Systems. When working with a group in which hybrids are suspected, one may choose to accept the hybrid challenge and attempt to work out the reticulating pattern of relationships (see above). On the other hand, one may well wish simply to achieve an estimate of phylogenetic relationships that is robust to the presence of undetected hybrids (i.e., in which the basic cladistic structure is likely to be correct). In such cases, one's choice of characters depends on the sorts of errors that one is willing to accept.

Using characters for which hybrids are expected to express conditions that are intermediate between their parents will result in the addition of hybrids to the intermediate nodes of the cladogram. Placed in this way, the relationships of the hybrid will not be apparent, but relationships among other taxa are not likely to be disrupted very profoundly. Intermediate hybrids also will not alter one's understanding of character evolution within the group because they will have little impact on the pattern of nested sets of derived characters that reflect phylogenetic order.

Working with characters for which hybrids express extreme character states will add taxa at the "margins" of a cladogram, thus giving an inflated notion of the extremes of character evolution within the group. Such hybrids will be proximate to the most derived parent, but

may give little signal of the special relationship to the other parent. This sort of hybrid's effect on relationships of the other taxa will depend on the number of synapomorphies that it shares with its least derived parent (i.e., the one with which it is not placed). If such characters are numerous, the hybrid may disrupt the nested pattern of derived states, and hence relationships, considerably (see Figure 3e).

Characters that ally hybrids with one parent or the other will lead one to be positively misled about sister group relationships (see Rieseberg and Soltis [1991] for an excellent review of such problems with specific regard to data from chloroplast DNA). The use of such character systems will be especially unsatisfactory in groups where hybridization is common: a phylogenetic hypothesis may tolerate only a few nonsensical sister pairs without losing credibility. This kind of outcome is also likely to be problematical when one is working at higher taxonomic levels. The improbable placement of a species or two is not likely to be as inflammatory as the counter-intuitive placement of a family or order due, for example, to plastid capture sometime in the distant past. Indeed, Rieseberg and Soltis (1991) argue that hybridization, coupled with the other peculiarities of organelle descent (e.g., no recombination, uniparental inheritance) may severely undermine the use of this character system alone.

Finally, characters for which hybrids are expected to express the derived states of both parents have the greatest potential for distorting relationships within the entire group due to potential shifts in placement of parental taxa. Patterns of character evolution will also be unclear due to the presence in one taxon of the derived character states that define distinct lineages.

In sum, in groups where hybridization is suspected, but cannot be resolved for whatever reason, using character systems for which hybrids will be scored as intermediate seems likely to yield cladograms with the least disruption to relationships of other taxa. When using the results of phylogenetic analyses based on other kinds of characters, one is well-advised to keep in mind the kinds of misrepresentations of relationships and of patterns of character evolution that are likely if hybrids are present. I would suggest, therefore, that an important function of cladograms based on morphological characters (for which hybrids are expected to be intermediate) is as a "reality check" on those developed from other kinds of characters. When such cladograms are incongruent with those based on morphology, hybridization should be considered as potentially distorting the former. Of course, when hybridization is not involved (and barring other sorts of biological phenomena that are likely to affect one character system or another in predictable ways), there is no reason to point to cladograms based on any one character system as particularly likely to mislead. Further, as described above, when hybridization is suspected, the use of multiple character systems can dramatically corroborate the hypothesis that hybridization has indeed occurred (see also Rieseberg and Soltis, 1991).

It should be noted that, for those cladists who are interested in patterns of character expression rather than the biological phenomena that underlie them, hybridization is only a problem in that analyses using different character systems will yield different cladograms (i.e., summaries of character patterns). Such "pattern cladists" would probably argue that the best approach is to combine data sets to yield a cladogram that best depicts all character data.

New Twists in Hybridization. As reported herein, there has been considerable progress toward understanding hybridization. However, a number of issues remain controversial, including the nature, frequency, and evolutionary significance of hybridization (see below). In addition, as is perhaps to be expected, the recent progress that has been achieved has not been without complications. Rather, a number of studies have yielded intriguing results that have actually complicated rather than clarified our understanding of hybrids and hybridization. Here, I will briefly describe two such complicating factors of which we have recently become aware.

First, a number of studies have shown that hybrid zones are much more permeable to organelles than to other signals of hybridity. When two lineages are hybridizing, one can usually describe a hybrid zone within which individuals exhibit evidence of hybrid origin in the form of intermediate morphologies, possession of the enzyme alleles characteristic of both lineages, etc. (see Barton and Hewitt, 1985, 1989). However, it is common to find that individuals that are far from the hybrid zone and that lack any other evidence of hybrid origin actually bear the organelles of the other lineage. For example, Lehman et al. (1991) found coyote mitochondria in animals that otherwise appear to be pure wolves. Ferris et al. (1983) and Dowling and Hoeh (1991) documented similar patterns in European mice and North American minnows, respectively. Wendel et al. (1991) have shown that one species of Australian *Gossypium* has the chloroplast DNA characteristic of members of an entirely different section of the genus, but lacks any other evidence of hybrid origin. Paige et al. (1991) showed that chloroplasts penetrate a hybrid zone between two cottonwood species such that those of one species appear in the otherwise apparently pure population of the other species, but not vice versa. Rieseberg and Soltis (1991) review this phenomenon in plants and argue that it is common. As alluded to in the section above on patterns expected for organelle characters, there are no doubt perfectly reasonable biological explanations for these observations. However, the pattern is disturbing because it means that research using different character systems will give incongruent results. It is hard enough to decide how to deal with hybrids when they consistently reflect their reticulate origin in all of their characters. The prospect of deciding how to handle "partial" or "segmental" hybrids is daunting. The evolutionary implications of such complex patterns of reticulation also merit contemplation.

Second, using techniques that permit discrimination of populations within parental species, multiple origins of several allopolyploid species have been demonstrated (Brochmann et al., 1992; Novak et al., 1991; Ownbey and McCollum, 1954; see also Stace, 1975). Assuming that hybridization is a fairly rare event and that polyploidization is at least as rare, it has usually been thought safe to assume that species that originated in this way had a single origin. Pragmatically, unless there has been a great deal of differentiation among populations of the progenitor species, this sort of *micropolyphyly* is likely to be of limited systematic importance. On the other hand, if the progenitor species are polytypic, then the independent hybrids between them may in fact look quite different. Similarly, wide-ranging progenitor species that independently give rise to hybrid allopolyploids in different parts of their ranges could wreak havoc with our understanding of historical biogeography.

In a sense, these complications merely magnify the problems caused by hybridization in general. Fundamentally, hybrids foul up the hierarchy of life because their histories are reticulating rather than divergent. They do not fit happily into phylogenies nor into the hierarchical system that we use to classify organisms. To the extent that there are "degrees" of hybridity (i.e., that organisms can be hybrids for some characters and not for others) and to the extent that hybrid taxa can be polyphyletic, these unhappy—but very real!—effects of hybrids are exacerbated.

PHYLOGENY, EVOLUTION, AND HYBRIDIZATION

What does the foregoing analysis of hybrids, their characters and cladistic behavior suggest about the larger-scale systematic and evolutionary roles of hybrids? First, in order to speculate about the phylogenetic significance of hybridization with any hope of accuracy, we need to confront the question of what kinds of hybrids we are dealing with and in what frequencies. Second, we must think clearly about how hybrids that have had eventful evolutionary histories (i.e., *derived* hybrids) will compare with the primary hybrids that have been the subject of this paper. Ideas about these two aspects of hybridization—even if tentative—

permit informed speculation about the phylogenetic impact of hybrids. Finally, does this examination of hybrids and their characters influence our thinking about the significance of hybridization in the progressive evolution of plants?

Frequency and Kinds of Hybrids. One can readily find statements in the literature about the commonness of hybrids and the origin of higher taxa via hybridization, but what do we really know about these issues? Most estimates of the incidence of hybridization among plants are based on the proportion of plant species thought to be allopolyploid (Goldblatt, 1979; Lewis, 1979; Stebbins, 1974). However, counts of polyploid species will overestimate the number of evolutionarily successful hybridization events because a single hybridization event followed by polyploidy and divergence may well account for the ploidy level of an entire species-rich lineage. Far better estimates of the incidence of phylogenetically significant hybridization will be possible once we have fairly detailed phylogenies for many groups of plants.

As noted previously, many of the speculations that have been made about the evolutionary history of hybridization are imprecise as to the nature of the entities that are hypothesized to have hybridized. What does it mean to suggest that many higher taxonomic groups may be of hybrid origin (see quote from Stebbins [1950] in the introduction)? Do individuals from different plant families hybridize with each other and do their descendants, as stabilized hybrid lineages (perhaps polyploid), then give rise to new families? Do entire genera or families reticulate such that the parental lineages are replaced by a single lineage of hybrid origin? Do individuals from reasonably closely related species hybridize and their descendants, as stabilized, perhaps polyploid, hybrid lineages, then give rise to new families or orders?

One approach to this problem is to assume that what is occurring at present provides a good estimate of what has happened in the past. Such a uniformitarian approach at least makes it possible to gather data on which to focus the discussion. The kinds and frequencies of hybridization events that are taking place at present are not sufficiently known to permit quantification. (In fact, I would urge that systematists take care to report the incidence and degree of relationship between hybridizing entities in the groups that they study so that we might amass such data; this same plea was made by Herb Wagner in 1968). Still, I think that a few speculations might be hazarded.

First, it makes little sense to identify hybridizing entities as higher than the species level. Even if two genera have hybridized, the phenomenon is more accurately described as individuals of species *x* from genus *a* hybridizing with individuals of species *y* from genus *b*. The potential exception presented by monotypic genera (or families) reduces to single species cases as well. At the other extreme, if all of the species in a polytypic genus were to be involved in a hybrid complex, it is likely that systematists would concur that this is a taxonomically problematic situation that should be treated as a polytypic species or species complex rather than as a genus with distinct species.

Second, my guess is that most systematists would agree that the discovery of a natural hybrid between species belonging to different higher level taxa (e.g., families) would have strong potential to yield a publication in one of the elite journals of science. From this, I would argue that most cases of hybridization are between close relatives (congeners) and that hybridization between distant relatives is rare (but see Robertson et al., 1991; Rieseberg and Soltis, 1991). However, given that geological time is extensive enough to have allowed for many rare events, we must still ask how rare a phenomenon must be before it can be judged unimportant. I do not know the answer.

Third, based again on what we know from the results of detailed monographic research on a variety of plant groups, it seems clear that hybridization is sufficiently rare that it

is noteworthy. However, it is also common enough that the description of putative hybrids is not normally thought to warrant publication on its own. On the geological time scale, this must amount to a very high incidence of hybridization. Of course, the more important frequency, as noted by Stebbins (1969), is much harder to estimate: once formed, how often do hybrids establish themselves as distinct lineages? Further, do these lineages, once established, have the same rates of speciation and extinction as taxa of divergent origin? We have, I would argue, few data that are of use in estimating these important rates. However, these issues can now be approached by using a combination of techniques to identify lineages that are of hybrid origin and by using a phylogenetic approach to compare these to closely related taxa of divergent origin. Abbott (1992) has suggested that hybridization involving invading plant species may offer special opportunities to study the factors that influence the establishment and spread of hybrid species.

My conclusion is that hybridization has been common in geological time and has almost always been between species that are closely related. If one does not accept that this pattern has been consistent throughout evolutionary history, then one must either argue that plants have differed in their propensity to hybridize over evolutionary time or that environmental conditions were differentially favorable for hybridization in the past. Although both may be possible, I believe that it is most conservative to accept the uniformitarian approach as a reasonable point of departure. What then do these ideas suggest about how lineages of ancient hybrid origin are likely to affect our modern attempts to understand plant phylogenies? One more piece of the hybrid puzzle must be considered before this question can be contemplated.

Derived Hybrids. The present paper has taken the simplifying approach of dealing with what I have called primary hybrids. However, it is likely that many hybrids have much more complicated histories, involving character evolution and/or divergence. Educated guesses about the impact of such derived hybrids must be framed in terms of the characters that these hybrids are likely to express. If hybrid and non-hybrid lineages have had similar rates of evolution, then derived hybrids should have much the same character patterns as primary hybrids except that, as evolution proceeds, hybrid and parental lineages alike will accumulate autapomorphies. As characters diverge in distinctive ways in one or more of these lineages, there should be progressively less evidence (in the form of synapomorphies) of the hybridization event. As a result, derived hybrid groups should be even more like normal derived taxa when studied using character systems for which apomorphy is expected. Using character systems for which hybrid intermediacy is expected, derived hybrids should continue to be intermediate between their parents, but will accumulate their own autapomorphies over evolutionary time. As for character systems for which primary hybrids share the derived features of both parents, this incongruent pattern should be blurred as evolution changes synapomorphies into autapomorphies and derived hybrids should behave more like divergently evolving taxa. This is precisely the pattern that is suggested by Rieseberg and Ellstrand's (1993) analysis of character patterns in hybrid species versus those in first generation hybrids. The former have considerably more extreme characters than the latter.

On the other hand, if a hybrid lineage has evolved (i.e., accumulated autapomorphies) faster than the non-hybrid lineages, this will exacerbate the cladistic behavior expected when hybrids have character states that are more extreme than either parent (Figure 3e). At worst, such hybrid lineages will be so highly autapomorphic that they may be recognized as higher level taxa equal in rank to a taxon that includes the other descendants of their progenitors, thus creating a propensity for paraphyletic groups. Consider, for example, the case of catostomid fish which are hypothesized to have evolved via a hybridization event between

two cypriniform fishes some 50 MYA (Uyeno and Smith, 1972). The non-hybrid taxa that descended from the lineages that hybridized to produce catostomids are presently placed in the family Cyprinidae, which is a paraphyletic group if this hypothesis is correct.

Thus, assuming that the uniformitarian approach taken above to deducing crude estimates of the kinds of hybridization that have taken place in the past is correct such that hybridization has indeed been mostly between close relatives, derived hybrids might not have much more impact on phylogenies than primary hybrids. We already know that cladistics cannot, in its present stage of development, produce the correct (i.e., reticulate) history of hybrids (but see above). Beyond this problem, a worse case scenario might involve a higher level cladistic analysis with three unfortunate characteristics: the hybrid lineage resulted from hybridization between non-sister taxa, all lineages that were present at the time of hybridization are still extant, and the characters used are such that the hybrid lineage combines the derived states of both parental lineages. This sort of situation is likely to be resolved with added homoplasy and considerable disruption of relationships of the non-hybrid taxa. However, as any of the conditions of the worse case analysis change, the solution should become less problematical. Hybridization between sister taxa reduces the scope of the problem to a three-lineage complex that is difficult to resolve. If one or more of the lineages that were present at the time of hybridization go extinct, the problem becomes much simpler (assuming that one has information only for extant taxa!). For example, if a hybrid and only one of its parental lineages remain, these are likely to be placed as sister taxa without distorting other relationships. Finally, as documented above, using other character systems should foul up the pattern of nested derived character states less dramatically, thus resulting in placement of the hybrid lineage with less homoplasy and less distortion of the relationships of other lineages.

In sum, I believe that the much heralded difficulties posed by hybrids for unraveling plant phylogenies (e.g., Cronquist, 1987) may have been overstated. I would like to suggest, in fact, that hybridization has been something of a scapegoat in botanical systematics. I think it likely that past problems in unraveling plant phylogenetic histories have had at least as much to do with using non-cladistic methods and recognizing taxa that are not strictly monophyletic as with hybridization. There are many circumstances under which hybrid lineages are likely to cause relatively few problems in cladistic analyses. These can be further minimized by using a combination of character systems to identify groups that are of hybrid origin. This latter biosystematic approach, combined with phylogenetics, should enhance our ability to document lineages that are of hybrid origin and thus to approach more rigorously the very interesting questions of relative rates of speciation and extinction in hybrid compared to divergently evolving lineages.

Evolutionary Significance of Hybridization. Those who have thought profoundly about hybrids and hybridization in plants seem mostly to have concluded that hybridization may be responsible for a proliferation of species that are ecologically and floristically significant, but that it is not a source of novel characters, of new adaptive modes, of evolutionary progress (Heiser, 1949, 1973; Stebbins, 1950; Wagner, 1968; Stebbins [1969] is a bit more sanguine about the evolutionary role of hybrids). In this view, hybridization simply reshuffles characters that were already present in the parental lineages. This may well be the case. However, I would like to suggest that this perspective may be biased by the traditional use in systematics of morphological characters and that it merits continuing reevaluation.

It has been rather thoroughly documented that, compared to their parents, hybrids are morphologically intermediate (Wagner [1968] provides an especially clear discussion of this point; see also Wilson [1992]). However, having studied the ways that other kinds of characters are expressed in hybrids compared to their parents, I am impressed that hybridiza-

tion can indeed generate novelties, with particularly good examples coming from plant chemistry. We are accustomed to thinking of plants interacting with their environments as morphological entities. However, many biotic interactions are mediated by plant chemistry, including relationships with pollinators, frugivores, seed predators, and especially herbivores. Is it not possible that some of the major facets of the evolution of plant biology might be owed to new chemical compounds resulting from hybridization? Reciprocally, might the evolution of novel plant compounds due to hybridization be partly responsible for the evolution and diversification of animals whose life cycles are strongly linked to plants? The results of Floate and Whitham (1993) would seem to refute this notion. These researchers have found that many hybrid plants (especially backcrosses) are more susceptible to herbivore attack than individuals of pure parentage. Breakdown of chemosynthetic pathways in the hybrids are implicated as partially responsible for leaving the hybrids poorly defended. To my mind, these intriguing results support the idea that hybrid plants may experience intense selection from the organisms associated with their parental species, suggesting that a hopeful monster might indeed be immediately successful. Even if hybrid chemotypes that confer resistance are exceedingly rare, they may be significant over evolutionary time.

Also suggestive of a significant evolutionary role for hybridization are the results of a number of detailed studies of the process of diploidization, particularly in polyploid fish (e.g., Uyeno and Smith, 1972; Allendorf and Thorgaard, 1984; Ferris, 1984). Research on the expression of isozyme loci in polyploid lineages commonly indicates that a significant number of loci have divergent functions or tissue specificities. High levels of gene silencing are also reported, which may be true silencing but may also reflect extreme divergence associated with new functions. Thus it seems clear that genomic duplication "frees" one pair of alleles to evolve, leaving the other to cover that locus's original function. To my knowledge, these patterns are not yet as clear for plants. However, diploid expression has been found in many ferns that seem clearly to be polyploids (Gastony, 1991; Haufler, 1989) and the same pattern has been found for putatively ancient polyploid scrophs (Elisens and Crawford, 1988). What are those homologous loci doing? A few studies have also suggested that hybrid taxa may have different tissue specificities for a variety of secondary plant compounds (e.g., Wyatt and Hunt, 1991). These are the sorts of changes that can profoundly alter the ecological roles played by a lineage of hybrid origin, producing, for example, different flower colors to present to pollinators and different leaf chemistry to present to herbivores. These observations support my contention that far from representing a mere reshuffling of characteristics, hybridization followed by polyploidy can permit rapid evolution in ways that are likely to be ecologically significant, even if not morphologically spectacular.

Finally, whatever has been true of the evolutionary significance of hybrids in the past may change as a result of human impact on the biosphere. At least three effects of the human diaspora and subsequent population and cultural growth are likely to favor hybrids. First, humans create disturbed habitats in which hybrids often do better than their parents (e.g., Stebbins, 1969). Second, by both active as well as passive dispersal, humans have moved numerous species of plants and animals to new habitats on a global scale. This may bring together lineages that have not experienced selection for isolating mechanisms, thus enhancing rates of hybridization (Abbott [1992] reviews the role of introduced species in hybridization). Finally, and in part as a result of the first two effects, human impact on the biosphere is causing the extinction of native species at heretofore unimaginable rates. Extinction creates disturbed communities that may be more vulnerable to invasion by hybrids. I concur with Abbott (1992) that efforts should be made to detect hybrids and set up long-term studies to follow their ecological and evolutionary progress. Given the pace of these anthropogenic changes in the biosphere, it may be possible to advance significantly our understanding of speciation via hybridization in just a few decades.

Beyond the impact of hybridization on plant evolution, Whitham and coworkers (e.g., Floate and Whitham, 1993; Whitham et al., 1994) have suggested that plant hybrid zones function as centers of diversity for phytophagous insects. Their "hybrid bridge hypothesis" (Floate and Whitham, 1993) suggests that plant hybrids may facilitate host shifting by any number of animals that rely on plants: herbivores, parasites, pathogens, perhaps even mutualists. They argue that this kind of shift, via the "bridges" created by hybrids, may be evolutionarily easier than a jump between host species. If this is indeed the case, then plant hybrids may be important as sites for diversification of organisms that rely on plants, as well as in their own right.

In sum, my feeling is that it is premature to dismiss hybridization as macroevolutionarily insignificant. Rather, we need to expand our understanding of the ways that characters are expressed in hybrids compared to their parents, and to integrate this information with plant ecology. Additional research on the expression and function of multiple alleles in polyploid taxa of hybrid origin will be useful for understanding the impact of hybridization on biological functions. We must also seize the opportunity (even if we lament its existence) to study evolution by hybridization as it takes place in our increasingly disturbed biosphere. Research in all of these areas must be placed in an explicit phylogenetic context in order to enable comparisons with taxa that are not of hybrid origin and to permit generalizations.

These approaches to systematic and evolutionary problems are very much in the intellectual tradition of biosystematics, whether or not one wishes to use the term or to identify with it. My guess is that the incidence of the term biosystematics in this volume will reflect the fact that biosystematics has largely ceded the leadership role in this intellectual domain. To maintain its vibrancy as an organization, the International Organization of Plant Biosystematists may well wish to recapture the flag by reclaiming its legitimate intellectual offspring.

LITERATURE CITED

Abbott, R. J. 1992. Plant invasions, interspecific hybridization and the evolution of new plant taxa. Trends Ecol. Evol. 7: 401–405.

Allendorf, F. W. and G. H. Thorgaard. 1984. Tetraploidy and the evolution of Salmonid fishes. Pp. 1–53 *in* B. J. Turner (ed.), Evolutionary Genetics of Fishes. Plenum Press, New York.

Alston, R. E. and B. L. Turner. 1963. Natural hybridization among four species of *Baptisia* (Leguminosae). Amer. J. Bot. 50: 159–173.

Anderson, E. 1949. Introgressive Hybridization. Wiley, New York.

Arnold, M. L., B. D. Bennett, and E. A. Zimmer. 1990. Natural hybridization between *Iris fulva* and *Iris hexagona*: Pattern of ribosomal DNA variation. Evolution 44: 1512–1521.

Arntzen, J. W. and G. P. Wallis. 1991. Restricted gene flow in a moving hybrid zone of the newts *Triturus cristatus* and *T. marmoratus* in Western France. Evolution 45: 805–826.

Averett, J. E., W. J. Hahn, P. E. Berry, and P. H. Raven. 1987. Flavonoids and flavonoid evolution in *Fuchsia* (Onagraceae). Amer. J. Bot. 73: 1525–1534.

Baker, R. J., S. K. Davis, R. D. Branley, M. J. Hamilton, and R. A. VanDen Bussche. 1989. Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies on a contact zone in the pocket gopher, *Geomys*. Evolution 43: 63–75.

Barton, N. H. and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual Rev. Ecol. Syst. 16: 113–148.

_____ and _____. 1989. Adaptation, speciation, and hybrid zones. Nature 341: 497–503.

Birky, C. W., Jr. 1983. The partitioning of cytoplasmic organelles at cell division. Int. Rev. Cytol. 15: 49–89.

Brochmann, C., P. S. Soltis, and D. E. Soltis. 1992. Recurrent formation and polyphyly of Nordic polyploids in *Draba* (Brassicaceae). Amer. J. Bot. 79: 673–688.

Burton, R. S. 1990. Hybrid breakdown in developmental time in the copepod *Tigriopus californicus*. Evolution 44: 1814–1822.

Cannon, W. A. 1909. Studies in heredity as illustrated by the trichomes of species and hybrids of *Juglans*, *Oenothera*, *Papaver*, and *Solanum*. Publ. Carnegie Inst. Wash. 117: 1–67.

Corriveau, J. L. and A. W. Coleman. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Amer. J. Bot. 75: 1443–1458.

Cronquist, A. 1987. A botanical critique of cladism. Bot. Rev. (Lancaster) 53: 1–52.

Densmore, L. D. I., J. W. Wright, and W. M. Brown. 1989. Mitochondrial-DNA analyses and the origin and relative age of parthenogenetic lizards (Genus *Cnemidophorus*) II. *C. neomexicanus* and the *C. tesselatus* complex. Evolution 43: 942–957.

DePamphilis, C. W. and R. Wyatt. 1989. Hybridization and introgression in buckeyes (*Aesculus*: Hippocastanceae): A review of the evidence and a hypothesis to explain long-distance gene flow. Syst. Bot. 14: 593–611.

Doebley, J. 1990. Molecular evidence for gene flow among *Zea* species. BioScience 40: 443–448.

Dorado, O., L. H. Rieseberg, and D. M. Arias. 1992. Chloroplast DNA introgression in southern California sunflowers. Evolution 46: 566–572.

Dowling, T. E. and W. R. Hoeh. 1991. The extent of introgression outside the contact zone between *Notropis cornutus* and *Notropis chrysocephalus* (Teleostei: Cyprinidae). Evolution 45: 944–956.

Doyle, J. J., J. L. Doyle, and A. H. D. Brown. 1990. A chloroplast-DNA phylogeny of the wild perennial relatives of soybean (*Glycine* subgenus *Glycine*): Congruence with morphological and crossing groups. Evolution 44: 371–389.

Elisens, W. J. and D. J. Crawford. 1988. Genetic variation and differentiation in the genus *Mabrya* (Scrophulariaceae–Antirrhineae): Systematic and evolutionary inferences. Amer. J. Bot. 75: 85–96.

Fahselt, D. and M. Ownbey. 1968. Chromatographic comparison of *Dicentra* species and hybrids. Amer. J. Bot. 55: 334–345.

Fedyk, S., W. Chetnicki, and A. Banaszek. 1991. Genetic differentiation of Polish populations of *Sorex araneus* L. III. Interchromosomal recombination in a hybrid zone. Evolution 45: 1384–1392.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27: 401–410.

Ferris, S. D. 1984. Tetraploidy and the evolution of the Catostomid fishes. Pp. 55–93 *in* B. J. Turner (ed.), Evolutionary Genetics of Fishes. Plenum Press, New York.

_____, R. D. Sage, C. M. Huang, J. T. Nielsen, U. Ritte, and A. C. Wilson. 1983. Flow of mitochondrial DNA across a species boundary. Proc. Natl. Acad. Sci. U.S.A. 80: 2290–2294.

Floate, K. D. and T. G. Whitham. 1993. The "hybrid bridge" hypothesis: Host shifting via plant hybrid swarms. Amer. Naturalist 141: 651–662.

Freeman, D. C., W. A. Turner, E. D. McArthur, and J. H. Graham. 1991. Characterization of a narrow hybrid zone between two subspecies of big sagebrush. Amer. J. Bot. 73: 805–815.

Fukuda, I. 1984. Chromosome banding and biosystematics. Pp. 97–116 *in* W. F. Grant (ed.), Plant Biosystematics. Academic Press, Canada.

Funk, V. A. 1985. Phylogenetic patterns and hybridization. Ann. Missouri Bot. Gard. 72: 681–715.

Gastony, G. J. 1991. Gene silencing in a polyploid homosporous fern: Paleopolyploidy revisited. Proc. Natl. Acad. Sci. U.S.A. 88: 1602–1605.

_____ and G. Yatskievych. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in Cheilanthoid ferns. Amer. J. Bot. 79: 716–722.

Goldblatt, P. 1979. Polyploidy in Angiosperms: Monocotyledons. Pp. 219–239 *in* W. H. Lewis (ed.), Polyploidy: Biological Relevance. Plenum Press, New York.

Grant, P. R. and B. R. Grant. 1992. Hybridization of bird species. Science 256: 193–197.

Grant, V. 1971. Plant Speciation. 2nd Edition. Columbia Univ. Press, New York.

Gyllenstein, U. 1992. Direct sequencing of *in vitro* amplified DNA. Pp. 45–60 *in* H. A. Erlich (ed.), PCR Technology: Principles and Applications for DNA Amplification. W. H. Freeman, New York.

Hagen, R. H. 1990. Population structure and host use in hybridizing subspecies of *Papilio glaucus* (Lepidoptera: Papilionidae). Evolution 44: 1914–1931.

Haufler, C. H. 1989. Towards a synthesis of evolutionary modes and mechanisms in homosporous Pteridophytes. Biochem. Syst. Ecol. 17: 109–115.

_____ and W. Zongren. 1991. Chromosomal analyses and the origin of allopolyploid *Polypodium virginianum* (Polypodiaceae). Amer. J. Bot. 78: 624–629.

Hein, J. 1990. Reconstructing evolution of sequences subject to recombination using parsimony. Math. Biosci. 98: 185–200.

_____. 1993. A heuristic method to reconstruct the history of sequences subject to recombination. J. Molec. Evol. 36: 396–405.

Heiser, C. B., Jr. 1949. Natural hybridization with particular reference to introgression. Bot. Rev. (Lancaster) 15: 645–687.

_____. 1973. Introgression re-examined. Bot. Rev. (Lancaster) 39: 347–366.

Hennig, W. 1966. Phylogenetic Systematics. Univ. of Illinois Press, Urbana, IL.

Hill, C. R. and P. R. Crane. 1982. Evolutionary cladistics and the origin of Angiosperms. Pp. 269–361 *in* K. A. Joysey and A. E. Friday (eds.), Problems of Phylogenetic Reconstruction. Academic Press, London.

Hillis, D. M., A. Larson, S. K. Davis, and E. A. Zimmer. 1990. Nucleic acids III: Sequencing. Pp. 318–370 *in* D. M. Hillis and C. Moritz (eds.), Molecular Systematics. Sinauer Associates, Sunderland, MA.

_____, C. Moritz, C. A. Porter, and R. J. Baker. 1991. Evidence for biased gene conversion in concerted evolution of ribosomal DNA. Science 251: 308–310.

Hubbs, C. L. 1955. Hybridization between fish species in nature. Syst. Zool. 4: 1–20.

Humphries, C. J. 1983. Primary data in hybrid analysis. Pp. 89–103 *in* N. I. Platnick and V. A. Funk (eds.), Advances in Cladistics: Proceedings of the Second Meeting of the Willi Hennig Society. Columbia Univ. Press, New York.

Jackson, R. C. 1984. Chromosome pairing in species and hybrids. Pp. 67–86 *in* W. F. Grant (ed.), Plant Biosystematics. Academic Press, Canada.

Jay, M., J. L. Fiasson, R. Lumaret, and P. Ardouin. 1989. Tetraploidy and habitat differentiation reflected by flavonoid patterns in *Dactylis glomerata* from Galicia (Spain). Biochem. Syst. Ecol. 17: 283–285.

Kim, I. and G. D. Carr. 1990. Cytogenetics and hybridization of *Portulaca* in Hawaii. Syst. Bot. 15: 370–377.

Lavin, M. and J. J. Doyle. 1991. Tribal relationships of *Sphinctospermum* (Leguminosae): Integration of traditional and chloroplast DNA data. Syst. Bot. 16: 162–172.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1985. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution 39: 1318–1326.

Lehman, N., A. Eisenhawer, K. Hansen, L. D. Mech, R. O. Peterson, P. J. P. Gogan, and R. K. Wayne. 1991. Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. Evolution 45: 104–119.

Levy, M. and D. A. Levin. 1975. The novel flavonoid chemistry and phylogenetic origin of *Phlox floridana*. Evolution 29: 487–499.

Lewis, W. H. 1979. Polyploidy in angiosperms: Dicotyledons. Pp. 241–268 *in* W. H. Lewis (ed.), Polyploidy: Biological Relevance. Plenum Press, New York.

Maddison, W. 1994. Phylogenetic histories within and among species. Pp. 273–287 in P. C. Hoch & A. G. Stephenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard.

McDade, L. A. 1990. Hybrids and phylogenetic systematics. I. Patterns of character expression in hybrids and their implications for cladistic analysis. Evolution 44: 1685–1700.

_____. 1992. Hybrids and phylogenetic systematics. II. The impact of hybrids on cladistic analysis. Evolution 46: 1329–1346.

Meagher, T. R. 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of most-likely male parents. Amer. Naturalist 128: 199–215.

Mears, J. A. 1979. Chemistry of polyploids: A summary with comments on *Parthenium* (Asteraceae–Ambrosiinae). Pp. 77–101 *in* W. H. Lewis (ed.), Polyploidy: Biological Relevance. Plenum Press, New York.

Miller, D. L. and R. J. Behnke. 1985. Two intergeneric hybrids from the Bonneville Basin, Utah. Copeia 1985: 509–515.

Murphy, R. W., F. C. McCollum, G. C. Gorman, and R. Thomas. 1984. Genetics of hybridizing populations of Puerto Rican *Sphaerodactylus*. J. Herpetol. 18: 93–105.

Neale, D. B. and R. R. Sederoff. 1988. Inheritance and evolution of conifer organelle genomes. Pp. 251–264 *in* J. W. Hanover and D. E. Keathley (eds.), Genetic Manipulation of Woody Plants. Plenum Press, New York.

Nelson, G. and N. I. Platnick. 1980. Multiple branching in cladograms: Two interpretations. Syst. Zool. 29: 86–91.

Ness, B. D., D. E. Soltis, and P. S. Soltis. 1990. An examination of polyploidy and putative introgression in *Calochortus* subsection *Nudi* (Liliaceae). Amer. J. Bot. 77: 1519–1531.

Novak, S. J., D. E. Soltis, and P. S. Soltis. 1991. Ownby's *Tragopogons*: 40 years later. Amer. J. Bot. 78: 1586–1600.

Ornduff, R., B. Bohm, and N. A. M. Saleh. 1973. Flavonoids of artificial interspecific hybrids of *Lasthenia*. Biochem. Syst. Ecol. 1: 147–151.

Ownbey, M. and G. D. McCollum. 1954. The chromosomes of *Tragopogon*. Rhodora 56: 7–21.

Paige, K. N., W. C. Capman, and P. Jenetten. 1991. Mitochondrial inheritance patterns across a cottonwood hybrid zone: Cytonuclear disequilibria and hybrid zone dynamics. Evolution 45: 1360–1369.

Patterson, C. 1982. Morphological characters and homology. Pp. 221–274 *in* K. A. Joysey and A. E. Friday (eds.), Problems of Phylogenetic Reconstruction. Academic Press, London.

Pipkin, S. B. 1959. Cytological evidence of introgression between *Drosophila* species in Panama. Ann. Missouri Bot. Gard. 59: 417–434.

Rajora, O. P. 1990. Marker allozyme genes and alleles for differentiation of *Populus deltoides*, *P. nigra*, *P. maximowiczii*, and their interspecific hybrids. Canad. J. Bot. 68: 990–998.

Rieseberg, L. H. 1990. Homoploid reticulate evolution in *Helianthus* (Asteraceae): Evidence from ribosomal genes. Amer. J. Bot. 78: 1218–1237.

_____, S. Beckstrom-Sternberg, and K. Doan. 1990a. *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius*. Proc. Natl. Acad. Sci. U.S.A. 87: 593–597.

_____, R. Carter, and S. Zona. 1990b. Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* species (Asteraceae). Evolution 44: 1498–1511.

_____ and N. C. Ellstrand. 1993. What can molecular and morphological markers tell us about plant hybridization? Crit. Rev. Plant Sci. 12: 213–241.

_____ and J. D. Morefield. 1994. Character expression, phylogenetic reconstruction, and the detection of reticulate evolution. Pp. 333–353 *in* P. C. Hoch & A. G. Stephenson (eds.), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard.

_____ and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Pl. 5: 65–84.

Robertson, K. R., J. B. Phipps, J. R. Rohrer, and P. G. Smith. 1991. A synopsis of genera in Maloideae (Rosaceae). Syst. Bot. 16: 376–394.

Sage, R. D. and R. K. Selander. 1979. Hybridization between species of the *Rana pipiens* complex in central Texas. Evolution 33: 1069–1088.

Schultz, R. J. 1979. Role of polyploidy in the evolution of fishes. Pp. 313–340 *in* W. H. Lewis (ed.), Polyploidy: Biological Relevance. Plenum Press, New York.

Seaman, F. C. and V. A. Funk. 1983. Cladistic analysis of complex natural products: Developing transformation series from Sesquiterpene Lactone data. Taxon 32: 1–27.

Sears, B. B. 1980. Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4: 233–255.

Short, L. L. 1972. Hybridization, taxonomy, and avian evolution. Ann. Missouri Bot. Gard. 59: 447–453.

Sites, J. W., D. M. Peccinini-Seale, C. Moritz, J. W. Wright, and W. M. Brown. 1990. The evolutionary history of parthenogenetic *Cnemidophorus lemniscatus* (Sauria, Teiidae). I. Evidence for a hybrid origin. Evolution 44: 906–921.

Soltis, D. E. and P. S. Soltis. 1989. Allopolyploid speciation in *Tragopogon*: Insights from chloroplast DNA. Amer. J. Bot. 76: 1119–1124.

Spolsky, C. and T. Uzzell. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. Proc. Natl. Acad. Sci. U.S.A. 81: 5802–5805.

Spring, O. and E. E. Schilling. 1989. Chemosystematic investigation of the annual species of *Helianthus* (Asteraceae). Biochem. Syst. Ecol. 17: 519–528.

Stace, C. A. 1975. Hybridization and the Flora of the British Isles. Academic Press, London.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York.

_____. 1969. The significance of hybridization for plant taxonomy and evolution. Taxon 18: 26–35.

_____. 1974. Flowering Plants: Evolution Above the Species Level. Belknap Press, Cambridge.

Sullivan, V. I., J. Neigel, and B. Miao. 1991. Bias in inheritance of chloroplast DNA and mechanisms of hybridization between wind- and insect-pollinated *Eupatorium* (Asteraceae). Amer. J. Bot. 78: 695–705.

Swofford, D. L. and G. J. Olsen. 1990. Phylogeny reconstruction. Pp. 411–501 *in* D. M. Hillis and C. Moritz (eds.), Molecular Systematics. Sinauer Associates, Sunderland, MA.

Talbert, L. E., J. F. Doebley, S. Larson, and V. L. Chandler. 1990. *Tripsacum andersonii* is a natural hybrid involving *Zea* and *Tripsacum*: Molecular evidence. Amer. J. Bot. 77: 722–726.

Tegelström, H. and H. P. Gelter. 1990. Haldane's Rule and sex biassed gene flow between two hybridizing flycatcher species (*Ficedula albicollis* and *F. hypoleuca*, Aves: Muscicapidae). Evolution 44: 2012–2021.

Uyeno, T. and G. R. Smith. 1972. Tetraploid origin of the karyotype of Catostomid fishes. Science 175: 644–646.

Vyas, D. K., C. Moritz, D. Peccinini-Seale, J. W. Wright, and W. M. Brown. 1990. The evolutionary history of parthenogenetic *Cnemidophorus lemniscatus* (Sauria: Teiidae). II. Maternal origin and age inferred from mitochondrial DNA analyses. Evolution 44: 922–932.

Wagner, W. H., Jr. 1962. Irregular morphological development in hybrid ferns. Phytomorphology 12: 87–100.

_____. 1968. Hybridization, taxonomy and evolution. Pp. 113–138 *in* V. H. Heywood (ed.), Modern Methods in Plant Taxonomy. Academic Press, London.

_____. 1971. Evolution of *Dryopteris* in relation to the Appalachians. *In* P. C. Holt (ed.), The Distributional History of the Southern Appalachians, Part II: Flora. Virginia Polytechn. Inst. State Univ. Res. Div. Monogr. 2: 147–192.

_____. 1980. The origin and philosophy of the groundplan-divergence method of cladistics. Syst. Bot. 5: 173–193.

Warwick, S. I., J. F. Bain, R. Wheatcroft, and B. K. Thompson. 1989. Hybridization and introgression in *Carduus nutans* and *C. acanthoides* reexamined. Syst. Bot. 14: 476–494.

Wendel, J. F., J. McD. Steward, and J. H. Rettig. 1991. Molecular evidence for homoploid reticulate evolution among Australian species of *Gossypium*. Evolution 45: 694–711.

Werth, C. R. 1989. The use of isozyme data for inferring ancestry of polyploid pteridophytes. Biochem. Syst. Ecol. 17: 117–130.

Whitham, T. G., P. A. Morrow, and B. M. Potts. 1994. Plant hybrid zones as centers of biodiversity: the herbivore community of two endemic Tasmanian eucalypts. Oecologia (in press).

Wiley, E. O. 1981. Phylogenetics: The Principles and Practice of Phylogenetic Systematics. Wiley, New York.

_____, D. Siegel-Causey, D. R. Brooks, and V. A. Funk. 1991. The Compleat Cladist: A Primer of Phylogenetic Procedures. Special Publ. 19, Museum of Natural History, University of Kansas, Lawrence, Kansas.

Wilson, P. 1992. On inferring hybridity from morphological intermediacy. Taxon 41: 11–23.

Wyatt, R. and D. M. Hunt. 1991. Hybridization in North American *Asclepias*. II. Flavonoid evidence. Syst. Bot. 16: 132–142.

CHARACTER EXPRESSION, PHYLOGENETIC RECONSTRUCTION, AND THE DETECTION OF RETICULATE EVOLUTION[1]

Loren H. Rieseberg[2] and James D. Morefield[3]

ABSTRACT. Reticulate evolution appears to play a prominent evolutionary role in many plant groups, yet the phylogenetic patterns of hybrids and their parents have rarely been studied in detail. In this paper, we test the effectiveness of parsimony criteria and probabilistic models for identifying artificially synthesized or previously documented homoploid hybrid species in *Gilia* and *Helianthus*, respectively, using a variety of different character sets. This combined phylogenetic and probabilistic approach is effective in terms of identifying the *Gilia* hybrid derivatives, suggesting that the assumptions underlying these methods are valid. However, the build-up of numerous morphological apomorphies in the *Helianthus* hybrid species results in a decrease rather than the predicted increase in parsimony for each of the hybrid hypotheses. Likewise, the character patterns produced are more similar to those predicted for random data than to those predicted for a hybrid origin. Chemical data performs considerably better than the morphological data, but the large increase in parsimony predicted is not realized. In contrast, the use of multiple gene trees is effective for identifying hybrid species in *Helianthus*. Theoretical considerations, however, suggest that many hybrid lineages will be cryptic to this approach as well, due to sampling error and the age of the reticulation event.

Reticulate evolution remains one of the most intractable problems for the field of phylogenetic systematics. That is, cladistic methods were designed to depict hierarchies (Hennig, 1966) and may therefore be inappropriate for the study of potentially hybridizing taxa. Yet, given the high frequency of hybridization in plants (Grant, 1981; Rieseberg and Wendel, 1993), this would have the effect of excluding cladistic analyses of relationships within and among many plant genera—clearly an unacceptable solution to the problem of reticulate evolution.

Practicing cladists have dealt with this problem in several ways (Funk, 1985). Many workers appear to have ignored the possibility of reticulate evolution. Whether this is because hybridization is considered to be rare and thus unimportant in "their group" of organisms or because of a lack of explicit procedures for treating hybrid lineages in phylogenetic analyses is not clear. Others have suggested that hybrids be identified prior to phylogenetic analysis and then excluded from further phylogenetic study (Wagner, 1969, 1983). A third group has suggested certain predictable observations for hybrid taxa in phylogenetic studies, which they believe may allow hybrid lineages to be identified by cladistic analyses (Nelson, 1983; Humphries, 1983; Funk, 1985). However, recent studies of the phylogenetic patterns of *Aphelandra* hybrids and their parents led McDade (1991, 1992) to predict that cladistics would not be an effective tool for identifying hybrids.

[1]This research was supported by NSF Grant BSR-9019872 to LHR and BSR-9000893 to JDM.
[2]Biology Department, Jordan Hall, Indiana University, Bloomington, IN 47405, U.S.A.
[3]Nevada Natural Heritage Program, 123 W. Nye Lane, Carson City, NV 89710, U.S.A.

Clearly, the first two approaches to dealing with the problem of reticulation are no longer tenable for most plant groups. The widespread occurrence of allopolyploidy in many plant groups has been known for more than half a century (Stebbins, 1950), and recent molecular evidence indicates that homoploid reticulate evolution may be pervasive in many plant groups as well (Rieseberg and Soltis, 1991; Rieseberg and Wendel, 1993). For example, Rieseberg and Soltis (1991) list 37 instances where one or more populations of a given species had the chloroplast DNA (cpDNA) genotype of another species, and most of the cases probably represent examples of homoploid reticulate evolution. It is also noteworthy that most of these cases were cryptic from a morphological and cytological standpoint and were not identified prior to phylogenetic analysis. Thus, the focus of this paper will be on the third approach, i.e., the identification of character state distributions diagnostic for hybrid lineages, which may then enable the detection of hybrids in phylogenetic studies.

Most previous studies of the phylogenetic patterns of hybrids and their parents have been based on first-generation hybrids (McDade, 1991, 1992), allopolyploids (Wagner, 1983), or theoretical constructs (Nelson, 1983). Although these investigations represent a valuable starting point for the study of reticulate parsimony, they are relatively unrealistic as models for the detection of homoploid hybrid species, which actually represent the end products of many generations of segregation and selection. Thus, the experimental models employed in this study will be stabilized homoploid hybrid derivatives, although the results should be applicable to recent hybrids and allopolyploids as well.

The objectives of this paper are to (1) test the effectiveness of parsimony criteria for identifying artificially synthesized or previously documented homoploid hybrid species in *Gilia* and *Helianthus*, respectively; (2) assess the utility of a variety of different character sets for hybrid detection; and (3) measure the relative likelihood of these hybrid hypotheses using probabilistic models (Table 1; Morefield, 1992).

Table 1. Expected frequencies for five null hypotheses, assuming 60 informative loci under Wagner parsimony. Invariant and uncertain relationships are considered uninformative (modified from Morefield, 1992).

	Relationship Supported			
Null Hypothesis	Invariant	Character State Shared with Parent 1	Character State Shared with Parent 2	Autapomorphy
1. Hybrid origin	--	30	30	0
2. Same as Parent 1	--	60	0	0
3. Same as Parent 2	--	0	60	0
4. Unrelated to either parent	--	0	0	60
5. Random data	20	20	20	20

MATERIALS AND METHODS

1. *Gilia*. In a series of elegant studies demonstrating the validity of the "recombinational speciation" model (Stebbins, 1957; Grant, 1958), Grant (1966a, 1966b) artificially synthesized three stabilized derivatives of the hybrid *Gilia malior* × *G. modocensis*. Of the three hybrid lineages or branches successfully produced, branch I and branch III each possessed a new combination of morphological and cytogenetic features (Grant, 1966a), whereas branch II reverted largely to the *G. modocensis* parent both morphologically and in terms of crossability (Grant, 1966b). The experiments were carried out over a period of 16 years (9–11 generations), and thus represent an excellent model system for studying character expression in hybrid species.

(i) *Morphological data*: Morphological characteristics of the parental species and three hybrid derivatives were scored by Grant (1966a, 1966b), and voucher specimens are deposited at RSA. For the purposes of the current study, all multistate characters were recoded to additive, binary components (Appendices 1–2).

2. *Helianthus*. The annual sunflowers of the genus *Helianthus* provide a rigorous test of the utility of a combined phylogenetic and probabilistic approach to the detection of reticulate evolution. This is because phylogenetic hypotheses based on morphological, chemical, and several types of macromolecular data are available for this group (Schilling and Heiser, 1981; Spring and Schilling, 1989; Rieseberg et al., 1991; Rieseberg, 1991). Moreover, hybridization has long been considered to play an important role in the evolution of this group (Heiser, 1947, 1949; Heiser et al., 1969), and recent molecular evidence indicates that at least 3 (*H. anomalus*, *H. deserticola*, and *H. paradoxus*) of the 12 species comprising *Helianthus* section *Helianthus* are stabilized diploid hybrid derivatives (Rieseberg et al., 1990; Rieseberg, 1991).

Nuclear ribosomal DNA (rDNA) variation provides the best evidence that these three species are of hybrid origin (Rieseberg et al., 1990; Rieseberg, 1991). Briefly, *H. anomalus*, *H. deserticola*, and *H. paradoxus* combine the rDNA repeat units of *H. annuus* and *H. petiolaris*—species whose rDNA repeats have been shown to differ by approximately 20 restriction site or length mutations. Chloroplast DNA evidence is concordant with this hypothesis, with *H. paradoxus* displaying the cpDNA haplotype of *H. annuus*, *H. deserticola* the cpDNA haplotype of *H. petiolaris*, and individuals of *H. anomalus* with the haplotype of either putative parental species (Rieseberg et al., 1991; Rieseberg, 1991).

(i) *Morphological data*: The morphological data set (Appendices 3–4) employed in this study was derived from four sources. Multistate characters 1–38 are recoded from Schilling and Heiser (1981). Multistate character 39 (stem mottled) was added to the character list after review of specimens at RSA-POM. Multistate characters 40–42 are from Spring and Schilling (1989), and multistate characters 43–54 are from Rogers et al. (1982). For the purposes of this study, all multistate characters were recoded to additive binary components, resulting in a total of 83 binary characters. Quantitative characters were included in the analysis because of the paucity of variable qualitative characters in sunflower, and because hybrid intermediacy can be observed for quantitative, but not qualitative, characters.

All characters (except those for oil and fatty acid content, pest resistance, and distribution of capitate glands) were re-scored based on specimens of all taxa deposited at RSA-POM, which include the collections of LHR. The re-scored data set was very similar to previous studies, except that it included all subspecific taxa as OTUs. This resulted in at least one change in scoring; a tap root (binary character 4) was scored as present in *H. niveus* subsp. *niveus* and *H. niveus* subsp. *tephrodes* and absent in *H. niveus* subsp. *canescens* in this study, whereas *H. niveus* was scored as lacking a tap root by Schilling and Heiser (1981). In addition, because multistate characters were recoded to additive, binary components, several characters had to be reformulated. Thus, leaf and phyllary pubescence (multistate characters 7 and 30, respectively), were re-scored in terms of percentage hair coverage. This resulted in some minor changes in scoring for these characters relative to Schilling and Heiser (1981).

(ii) *Chemical data*: The chemical data set (Appendix 5) employed in this study was derived from a series of investigations of the sesquiterpene lactone (SQL) chemistry of the genus *Helianthus* by Spring and Schilling (1989, 1991). Compounds were scored as present only if they occurred in significant quantities. The compound with retention time of 0.38 (Spring and Schilling, 1989) was not included because it was not present in significant amounts in any of the annual sunflowers.

(iii) *Molecular data*. Chloroplast DNA (Rieseberg et al., 1991) and nuclear rDNA (Rieseberg, 1991) data sets for section *Helianthus* were combined for the present study (Appendix 6). A single representative population was chosen for each taxon in order to simplify the molecular data set and make it more directly comparable to the morphological and chemical data sets. Again, all multistate characters (certain rDNA length mutations) were recoded to additive binary components. For the putative hybrid species, which combined the rDNA repeat units of their parents, a site or length mutation was scored as present if it occurred in one or both parental repeat units. A similar method of scoring was employed for *H. anomalus* cpDNA because both parental cpDNAs occurred within populations of the species.

3. *Cladistic Analyses*. Each of the data sets was analyzed using Wagner parsimony methods implemented by PAUP version 3.0 (Swofford, 1991). The Branch and Bound option was used to search for the most parsimonious trees. Consistency indices for each analysis were calculated excluding autapomorphies. In addition, a "strict" consensus tree (Rohlf, 1982) was derived for each analysis using PAUP.

To determine the effects of having more than one hybrid species in each analysis, phylogenetic analyses were also conducted where only one of the three hybrid species was included in each data set. Because of the small size of the *Gilia* data set, however, it was possible to exclude only one of the three hybrid lineages at a time. This turned out not to be a problem, because one of the three lineages (branch II) is similar to *G. modocensis*, and its presence or absence did not affect the results.

Trees for the *Gilia* data set were unrooted, whereas trees produced by the *Helianthus* data sets were rooted using the outgroup method (Watrous and Wheeler, 1981). Outgroup species for the morphological and SQL data sets were *H. agrestes*, *H. atrorubens*, *H. maximiliani*, and *H. porteri*. *Helianthus atrorubens* and *H. maximiliani* are perennial members of sect. *Divaricati* (Schilling and Heiser, 1981). *Helianthus porteri* is an annual species, also of sect. *Divaricati*. *Helianthus agrestes*, the only other annual sunflower in the genus (outside of section *Helianthus*), is placed in its own section (Schilling and Heiser, 1981). *Helianthus atrorubens*, *H. maximiliani*, and *H. porteri* were also employed as outgroups to root the molecular-based phylogenetic tree. Chloroplast DNA and rDNA data were not available for *H. agrestes*.

4. *Reticulate Parsimony*. To determine the increase or decrease in parsimony required by each hybrid hypothesis, as well as the percentage of terminal apomorphies in the putative hybrids that can be explained by the each hybrid hypothesis, we employed the computer program RETICLAD (Morefield, 1992). RETICLAD tests reticulation events between terminal taxa only; hybridization between internal nodes (hypothetical OTUs) cannot be analyzed. A complete description and copy of RETICLAD can be obtained upon request from JDM.

In order to implement RETICLAD, each of the data sets employed above (RETICLAD requires binary characters) were modified to include terminal branch lengths derived from the Wagner parsimony analysis. For each data set, all possible three-taxon combinations were then analyzed to find the most parsimonious hybrid hypotheses.

5. *Probabilistic Models*. RETICLAD also tests the character state distribution of the putative hybrid and its parents against expected frequencies for five null hypotheses (Table 1). Thus, for each data set the relative likelihoods of the most parsimonious hybrid hypotheses were tested using RETICLAD.

RESULTS

1. *Gilia.* Wagner parsimony analysis of the 12-character (21 binary character) morphological data set (Appendices 1–2) resulted in two equally parsimonious 26-step trees (Figure 1). The consistency index was 0.69. Analysis with RETICLAD indicated that tree length was reduced by 4 steps for the branch I hybrid hypothesis, 1 step for the branch II hybrid hypothesis, and 0 steps for the branch III hybrid hypothesis (Table 2).

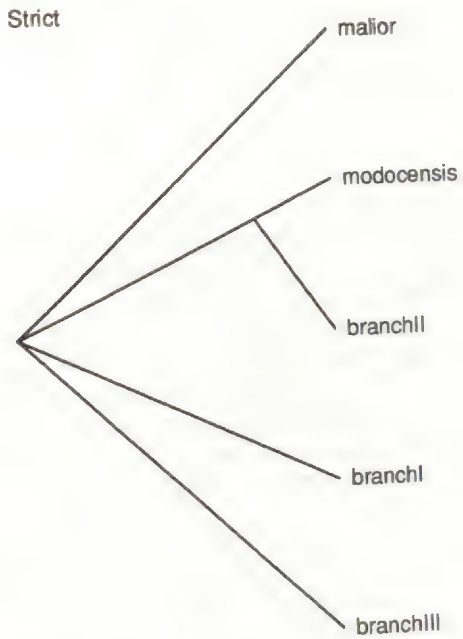


Figure 1. Unrooted consensus tree for *Gilia modocensis, G. malior,* and their three hybrid derivatives, based on morphological data.

To determine whether the production of three hybrid lineages from the same two parents was affecting these results, cladistic analyses of the same data set, except for the exclusion of branch I and branch III, respectively, were performed. Both analyses resulted in single 22-step most parsimonious trees with consistency indices of 0.88. Analysis with RETICLAD indicated an increase in parsimony of six steps if either branch I or branch III was postulated to be of hybrid origin, and four steps for the branch II hybrid hypothesis, regardless of whether branch I or branch III were excluded from the analysis (Table 2).

The character state distribution of the three hybrid lineages and their parents differed significantly from expected frequency distributions for all but the hybrid origin hypothesis. Nevertheless, even the hybrid origin hypothesis could be excluded ($p < 0.01$) for branch II. This should not be considered a surprising finding, however, given that branch II largely reverted to one of the parental species, *G. modocensis* (Grant, 1966b).

Thus, the reticulate parsimony approach was effective in diagnosing the hybrid origin and parentage of the artificially synthesized *Gilia* hybrids, although a dramatic increase in parsimony could be obtained only by removing either branch I or branch III from the phylogenetic analysis prior to analysis with RETICLAD.

Table 2. Statistics for artificially synthesized *Gilia* hybrid derivatives based on morphological data. Numbers in parentheses are results from phylogenetic analyses where branch I or branch III was excluded.

malior × *modocensis*	Change in Tree Length	Percent Hybrid Apomorphies Explained by Reticulation	Hybrid Origin	Chi-square Statistic: Random Data	Same as *malior*	Same as *modocensis*	Unrelated
F_9, branch I	-4.0 (-6.0)	100.0 (100.0)	0.8	18.8**	295.2**	131.2**	436.0**
F_{11}, branch II	-1.0 (-4.0)	50.0 (80.0)	11.4*	36.0**	595.4**	20.8**	625.0**
F_{10}, branch III	0.0 (-6.0)	0.0 (100.0)	0.8	18.8**	295.2**	131.2**	436.0**

* $p < 0.01$ ** $p < 0.001$

2. *Helianthus*.

(i) *Morphological Data*: Cladistic analysis of the 54-character (83 binary character) morphological data set (Appendices 3–4) generated 39 equally parsimonious 212-step trees (Figure 2) with a consistency index of 0.35. Analysis of all possible three taxon combinations with RETICLAD revealed that the most parsimonious hybrid hypotheses were not those originally postulated based on molecular data (Rieseberg, 1991), but rather involved the hybrid origin of *H. neglectus* from three possible sets of parents (Table 3). However, all three hybrid origin hypotheses involving *H. neglectus* can be excluded statistically ($p < 0.001$) based on their character state distributions. It is noteworthy that *H. neglectus* had previously been postulated to be of hybrid origin between *H. annuus* and *H. petiolaris* on morphological grounds (Heiser et al., 1969), but molecular evidence indicates that it is actually a recent derivative of *H. petiolaris* (Rieseberg et al., 1990).

The morphological data performed extremely poorly in terms of identifying the hybrid species previously postulated based on molecular data. In fact, these hybrid hypotheses actually required an increase in tree length of 5–11 steps, rather than the expected increase in parsimony (Table 4), and could be easily excluded statistically ($p < 0.001$).

To determine whether the production of three hybrid species from the same two parents was affecting our results, cladistic analyses of the same data set was performed, except that only one of the three hybrid species was included in each analysis (tree length and consistency index are in parentheses): *anomalus* (199, 0.36), *deserticola* (194, 0.36), and *paradoxus* (194, 0.37). However, analysis with RETICLAD indicated that the hybrid hypotheses still were not parsimonious (Table 4).

(ii) *Sesquiterpene Lactone Chemistry*: Wagner parsimony analysis of the 52-character SQL data set (Appendix 5) resulted in the production of over 100 equally parsimonious 63-step trees (Figure 3) with a consistency index of 0.55. Analysis of all possible three taxon combinations with RETICLAD revealed that the most parsimonious hybrid hypotheses were not those originally postulated based on molecular data (Rieseberg, 1991), but rather involved the hybrid origin of *H. deserticola* from two different sets of parents (Table 5). These hybrid hypotheses cannot be excluded statistically, nor is it possible to exclude the alternative null hypothesis that *H. deserticola* has the same SQL profile as *H. argophyllus* (Table 5).

The SQL data performed somewhat better than the morphological data in terms of identifying the sunflower hybrid species. Nonetheless, the hybrid hypotheses for *H. anomalus* and *H. paradoxus* still required an increase in tree length of 1 step rather than the predicted increase in parsimony (Table 6), although the *H. paradoxus* hybrid hypothesis could not be

excluded statistically. In contrast, the hypothesized hybrid origin of *H. deserticola* from *H. annuus* and *H. petiolaris* did result in a 1-step increase in parsimony, but was less likely from a statistical standpoint ($p < 0.001$).

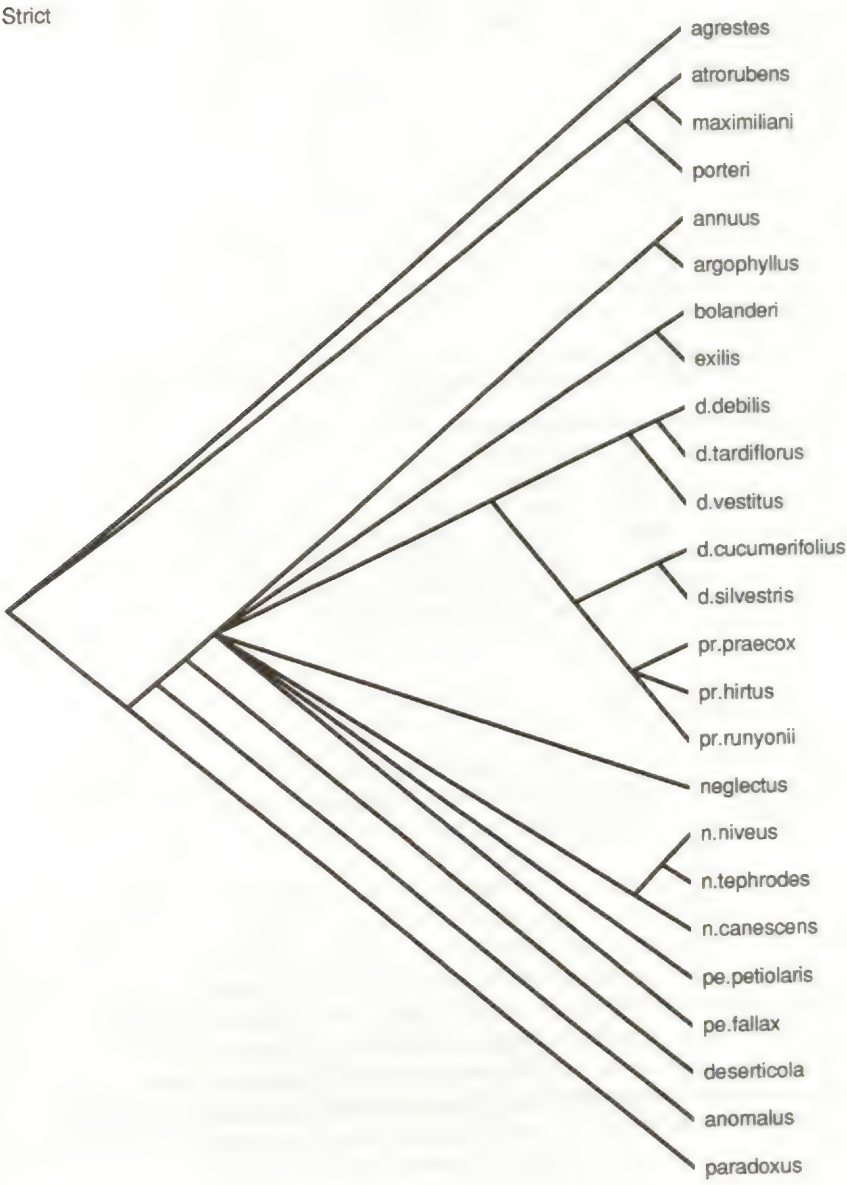


Figure 2. Strict consensus tree for *Helianthus* section *Helianthus*, based on morphological data.

Cladistic analyses of the same data set were performed, except that only one of the three hybrid species was included in each analysis (tree length and consistency index are in

Table 3. Most parsimonious hybrid hypotheses in *Helianthus* based on morphological data.

Hybrid Hypothesis	Change in Tree Length	Percent Hybrid Apomorphies Explained by Reticulation	Chi-square Statistic: Hybrid Origin	Random Data	Same as Parent 1	Same as Parent 2	Unrelated
annuus × *petiolaris*[1] => *neglectus*	-5.0	63.0	19.0**	79.1**	366.7**	151.0**	484.4**
argophyllus × *petiolaris*[1] => *neglectus*	-5.0	63.0	20.5**	66.8**	609.9**	185.0**	763.3**
argophyllus × *paradoxus* => *neglectus*	-5.0	63.0	18.6**	31.5**	605.8**	914.0**	1495.0**

[1]subsp. *fallax* most parsimonious parent. * $p < 0.01$ ** $p < 0.001$

Table 4. Statistics for *Helianthus* stabilized hybrid derivatives based on morphological data. Numbers in parentheses are results from phylogenetic analyses where only one of the three putative hybrid species was included.

annuus × *petiolaris*	Change in Tree Length	Percent Hybrid Apomorphies Explained by Reticulation	Chi-square Statistic: Hybrid Origin	Random Data	Same as *annuus*	Same as *petiolaris*	Unrelated
anomalus[1]	+11.0 (+6)	0.0 (0)	523.1**	24.7**	1052.4**	555.1**	555.8**
deserticola[1]	+5.0 (+1)	0.0 (0)	105.1**	51.9**	134.5**	558.8**	514.8**
paradoxus[1]	+11.0 (+11)	0.0 (0)	587.1**	30.0**	1054.3**	690.8**	560.4**

[1]subsp. *fallax* most parsimonious parent. * $p < 0.01$ ** $p < 0.001$

parentheses): *anomalus* (58, 0.55), *deserticola* (61, 0.55), and *paradoxus* (56, 0.57). However, analysis with RETICLAD indicated that the hybrid hypotheses resulted in the same changes in parsimony observed for the original data set (Table 6).

(iii) *Chloroplast DNA and nuclear ribosomal DNA data*: Wagner parsimony analysis of the 94-character molecular data set (Appendix 6) produced six equally parsimonious 127-step trees (Figure 4) with a consistency index of 0.58. Analysis of all possible three taxon combinations with RETICLAD revealed that all three of the most parsimonious hybrid hypotheses involved the origin of *Helianthus paradoxus* (Table 7). One of the sets of parents (*H. annuus* and *H. petiolaris*) was originally postulated based on visual inspection of the molecular data (Rieseberg, 1991), whereas the other two sets of parents involved *H. annuus* and one of the other putative hybrid derivatives. None of the three most parsimonious hybrid hypotheses could be excluded statistically.

The molecular data performed somewhat better than either the morphological or SQL data in terms of identifying the previously documented hybrid species in that none of the three putative hybrids required a decrease in parsimony (Table 6). Nonetheless, the individual hybrid hypotheses do not result in a great increase in parsimony. This is probably because all three hybrid species were derived from the same two parents and thus have almost identical cpDNA and rDNA genotypes. To correct this problem, cladistic analyses of the same data set

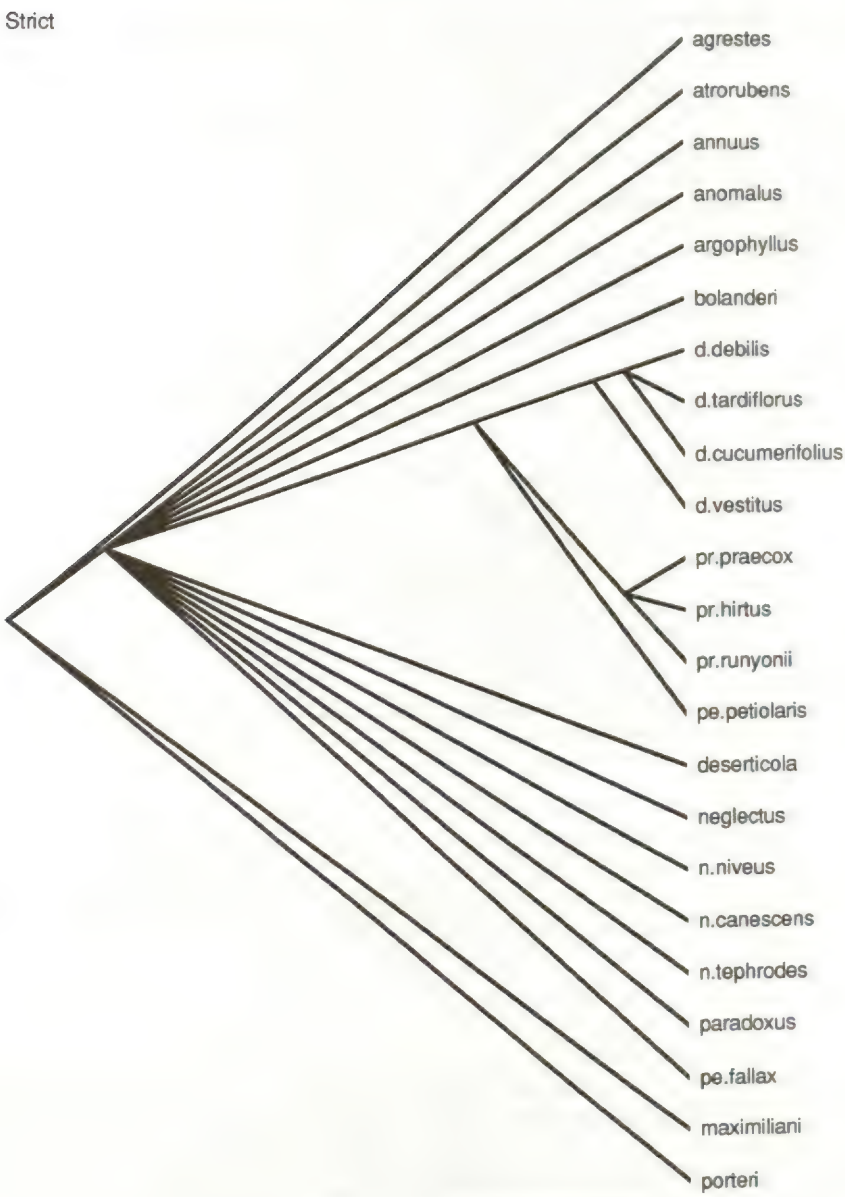


Figure 3. Strict consensus tree for *Helianthus* section *Helianthus*, based on sesquiterpene lactone chemistry.

were performed, except that only one of the three hybrid species was included in each analysis (tree length and consistency index are in parentheses): *anomalus* (114, 0.62), *deserticola* (120, 0.61), and *paradoxus* (120, 0.59). Analysis with RETICLAD now indicated a large increase in parsimony for each of the hybrid hypotheses (6–7 steps). In fact, the increase in parsimony observed was 3–4 steps greater than for any other hybrid hypothesis.

Table 5. Most parsimonious hybrid hypotheses in *Helianthus* based on sesquiterpene lactone data.

Hybrid Hypothesis	Change in Tree Length	Percent Hybrid Apomorphies Explained by Reticulation	Chi-square Statistic: Hybrid Origin	Random Data	Same as Parent 1	Same as Parent 2	Unrelated
anomalus × *argophyllus* => *deserticola*	-4.0	80.0	2.6	76.7**	37.6**	4.3	11.3*
argophyllus × *praecox* => *deserticola*	-4.0	80.0	8.8	46.2**	4.3	212.1**	212.1**

*$p < 0.01$ **$p < 0.001$

Table 6. Statistics for *Helianthus* stabilized hybrid derivatives based on sesquiterpene lactone data. Numbers in parentheses are results from phylogenetic analyses where only one of the three putative hybrid species was included.

annuus × *petiolaris*	Change in Tree Length	Percent Hybrid Apomorphies Explained by Reticulation	Chi-square Statistic: Hybrid Origin	Random Data	Same as *annuus*	Same as *petiolaris*	Unrelated
anomalus[2]	+1.0 (+1.0)	0.0 (0.0)	19.4**	67.2**	55.4**	28.8**	44.0**
deserticola[1]	-1.0 (-1.0)	20.0 (20.0)	34.0**	75.3**	70.4**	43.6**	43.6**
paradoxus[2]	+1.0 (+1.0)	0.0 (0.0)	3.9	82.2**	79.4**	11.0*	87.1**

[1]subsp. *fallax* most parsimonious parent. [2]subsp. *petiolaris* most parsimonious parent. *$p < 0.01$ **$p < 0.001$

Statistical analyses of the molecular data revealed that for each of the putative hybrid species, all hypotheses but the hybrid hypothesis could be excluded statistically. However, the build-up of rDNA and cpDNA autapomorphies in *H. deserticola* (the most ancient of the three hybrid species), subsequent to its origin (Rieseberg, 1991), makes its character state distribution a statistically poor fit for the hybrid hypothesis as well.

DISCUSSION

1. Limitations. As discussed by Morefield (1992), there are several limitations to the approach employed here to detect hybrid lineages, including: (1) The frequency distributions for the five null hypotheses represent extreme expectations, and therefore the statistics derived from them should be viewed as relative rather than absolute values for comparisons among hypotheses; (2) it may not be possible to determine whether a taxon is a hybrid derivative of two species or an ancestor to both because similar character state distributions are predicted for both situations (Funk, 1985); (3) because a variety of evolutionary processes other than hybridization are known to cause character conflict in phylogenetic trees (Avise, 1989), RETICLAD should be viewed as a tool to identify and evaluate the relative likelihood of a particular hybrid hypothesis rather than to prove or disprove hybridity (Morefield, 1992); and (4) the use of parsimony criteria to detect hybridity breaks down when more than one hybrid is derived from the same two parents. This is unfortunate because diploid hybrid speciation, unlike allopolyploidy, is often likely to result in the production of several different

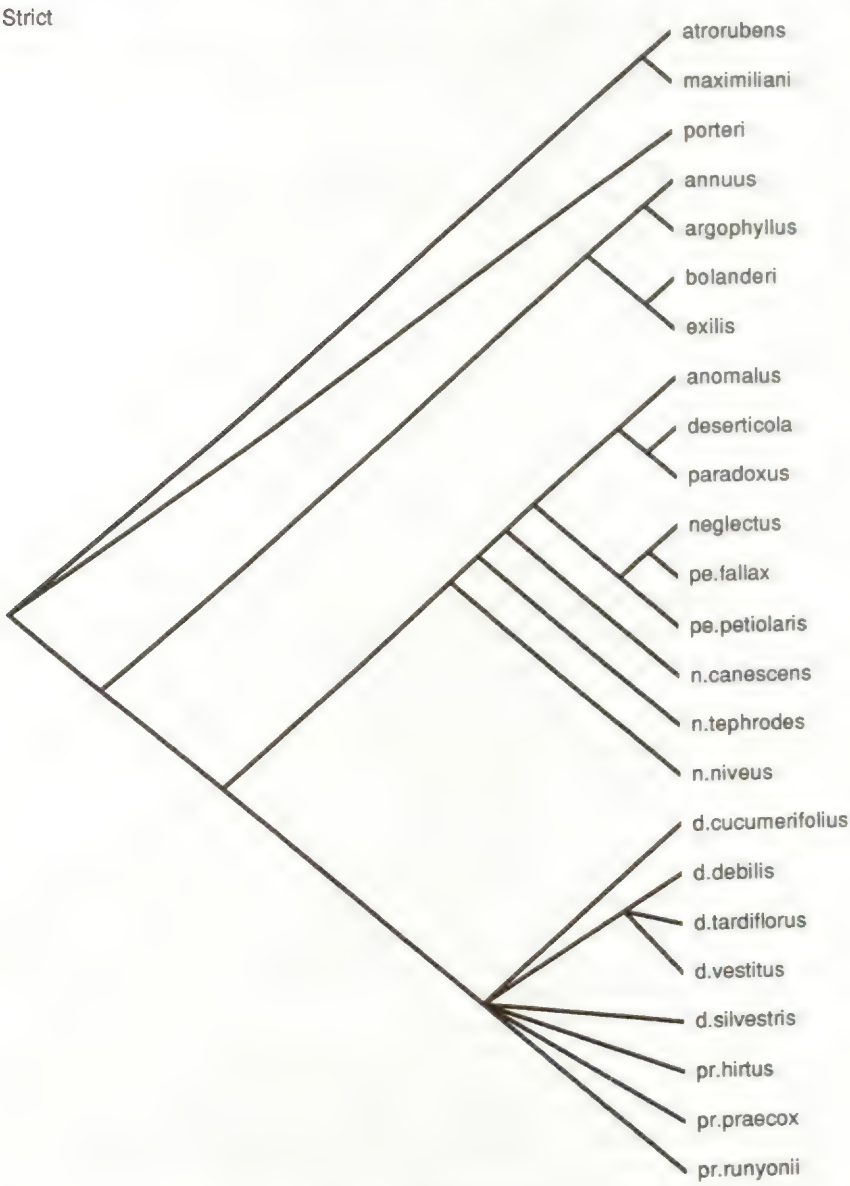


Figure 4. Strict consensus tree for *Helianthus* section *Helianthus*, based on cpDNA and rDNA data.

diploid hybrid species from the same two parents such as has been observed for *Helianthus* (Rieseberg et al., 1993). However, the presence of more than one hybrid derivative from a pair of parental species does not affect statistical tests based on expected character state frequencies. Thus, when more than one hybrid derivative from a pair of parents is anticipated, it may be best to search for the most likely hybrid hypotheses as well as the most parsimonious ones, and then exclude all but one of the hybrids identified in performing further analyses.

Table 7. Most parsimonious hybrid hypotheses in *Helianthus* based on cpDNA and nuclear rDNA data. The two subspecies of *H. petiolaris* represent equally parsimonious parents of the three hybrid species. Numbers in parentheses are results from phylogenetic analyses where only one of the three putative hybrid species was included.

Hybrid Hypothesis	Change in Tree Length	Percent Hybrid Apomorphies Explained by Reticulation	Chi-square Statistic				
			Hybrid Origin	Random Data	Same as Parent 1	Same as Parent 2	Unrelated
annuus × *petiolaris* => *paradoxus*	-3.0 (-7.0)	60.0 (78.0)	8.3	115.3**	256.5**	354.7**	602.2**
annuus × *petiolaris* => *deserticola*	-2.0 (-7.0)	33.0 (64.0)	34.9**	105.7**	558.3**	165.1**	660.6**
annuus × *petiolaris* => *anomalus*	0.0 (-6.0)	0.0 (100.0)	2.7	128.1**	522.1**	130.7**	664.0**
annuus × *deserticola* => *paradoxus*	-3.0	60.0	8.4	135.4**	257.7**	175.5**	422.2**
annuus × *anomalous* => *paradoxus*	-3.0	60.0	10.2*	158.2**	259.4**	60.7**	306.2**

* $p < 0.01$ ** $p < 0.001$

Regardless of these limitations, it is possible to make some significant, although perhaps unsurprising, conclusions about character expression in hybrids and their parents, and the use of parsimony as a criterion for detecting hybrid lineages.

2. Hybrid Character Expression. Analysis of the *Gilia* data set with RETICLAD demonstrates that the basic assumptions (Morefield, 1992) underlying the use of parsimony and probabilistic models for the detection of hybridity are valid. Both the character state distribution and the large increase in parsimony predicted for hybrids were observed for the two morphologically distinctive *Gilia* hybrid lineages. Even branch II, which had largely reverted back to *G. modocensis*, exhibited some features characteristic of hybrid lineages. Thus, the analytical methods employed here do have the potential to increase the likelihood of detection of recent hybrids and allopolyploids in phylogenetic studies. However, these conclusions must be considered preliminary because other closely related Gilias were not included in the study and have the potential to confound the results.

In contrast, the *Helianthus* hybrid derivatives possessed numerous morphological apomorphies, resulting in a large decrease in parsimony, as well as in character patterns more similar to random data than to those predicted for a hybrid origin. This poor result is probably due to the strong selection pressures the stabilized hybrid derivatives have been subjected to after the initial hybridization event, perhaps leading to the fairly rapid fixation of autapomorphies in the hybrids.

This hypothesis is supported by ecological data. *Helianthus anomalus* and *H. deserticola* are rare, xerically adapted sunflowers, endemic to sand dune and swale habitats in the desert regions of the southwestern United States, whereas *H. paradoxus* occurs in brackish, saline, marshy areas in west Texas. Both of these habitats are quite distinct from that of *H. annuus* (heavy soils that are wet in the spring but dry by midsummer) and *H. petiolaris* (dry, sandy soils), and would be predicted to result in a number of unique adaptations or autapomorphies in the hybrid species. For example, *H. paradoxus* is almost completely glabrous, a condition that characterizes many saline tolerant plants, whereas *H. annuus* and *H. petiolaris* exhibit varying degrees of pubescence.

The SQL data performed better than the morphological data set, perhaps reflecting lower selection pressures on these characters or a less complex genetic basis. Nonetheless, a large increase in parsimony was not achieved by invoking the hybrid hypotheses. Of the five null hypotheses, however, the hybrid origin hypothesis was the most likely statistically in each instance.

If *Helianthus* represents a reliable model for evolution in other plant groups, the poor performance of the morphological and chemical data for identifying hybrid sunflower species might be used as evidence to suggest that these types of data are unlikely to be particularly useful for detecting homoploid hybrid species, regardless of the analytical methods used. Alternatively, it might be argued that the original identification of these taxa as hybrids (Rieseberg, 1991) is invalid, and that the morphological and chemical data are providing a more reliable estimation of relationships than the molecular data. A possible alternative hypothesis, for example, is that the three hybrid species are actually ancestral to their putative parents (J. Doebley, pers. comm.). In this scenario, the ancestor of the section *Helianthus* would have to be polymorphic for the two highly divergent rDNA repeat units of *H. annuus* and *H. petiolaris*, as well as for the cpDNAs of the two species. This might occur via a duplication of the ribosomal RNA gene family in the ancestral species followed by rapid divergence of the duplicated cistrons. The degree of cpDNA differentiation between *H. annuus* and *H. petiolaris* is very low, and similar levels of divergence have been observed among populations of other plant species (reviewed in Soltis, Soltis, and Milligan, 1992). Thus, the maintenance of cpDNA polymorphisms within the ancestral species is not implausible. The next step in the scenario would require the loss of the *annuus* rDNA repeat and cpDNA haplotype in the *petiolaris* lineage and the loss of the *petiolaris* rDNA repeat and cpDNA haplotype in the *annuus* lineage, whereas both rDNA repeat types and one or both plastome types would have to be maintained in *H. anomalus*, *H. paradoxus*, and *H. deserticola* (see Rieseberg, 1991, Figure 8). There is a fatal flaw with this "polymorphic ancestor hypothesis," however, which is revealed when the other species of section *Helianthus* are considered in the light of this scenario. That is, clades placed basal in the rDNA phylogenetic tree (Rieseberg, 1991, Figure 8) would require a minimum of ten convergent mutations in order to be derived from the polymorphic ancestor. Alternatively, a minimum of two independent losses of the *petiolaris* rDNA repeat type and four independent losses of the *annuus* rDNA repeat type would be necessary. Thus, the polymorphic ancestor hypothesis cannot be considered a very parsimonious alternative to the hybrid origin hypothesis.

Clearly, the gene tree data were most effective in terms of detecting reticulate evolution. All three hybrid hypotheses showed large increases in parsimony if analyzed individually, and the hybrid origin hypothesis was statistically more likely than the other null hypotheses in each instance. Nonetheless, as was shown for *H. deserticola*, the more ancient the reticulation event, the less effective the tree approach will be (or for that manner any other line of evidence) due to the build-up of autapomorphies. Likewise, if the molecular data set had included all intraspecific cpDNA and rDNA polymorphism reported for *Helianthus* (Rieseberg et al., 1991; Rieseberg et al., 1990), it undoubtedly would have performed less effectively than reported here. Furthermore, with gene tree data, reticulation events will often be missed because of the low number of gene trees sampled in most studies. For example, five independent gene trees would be required to achieve 95% confidence that a particular reticulation event will be detected. And these calculations are based on the unwarranted assumption that a hybrid species will retain equivalent proportions of its parent's genomes (Rieseberg et al., 1993). Thus, the high number of gene trees required for statistically confident detection of reticulation may render this approach impractical for most systematic studies as well.

3. Effect of Hybrids on Tree Topology. The effects of hybrids on tree topology have long been a major concern of cladists (e.g., Bremer and Wanntorp, 1979; Nelson, 1983). Theoretical studies have suggested that hybrids will be likely not only to increase levels of homoplasy, but also to distort phylogenetic relationships among non-hybrid species (Humphries, 1983). The impact of the *Helianthus* hybrid species on homoplasy levels has been described previously (a large increase in homoplasy was observed; Rieseberg, 1991), but the effect of these hybrids on tree topology was not discussed. A secondary result of this study was the observation that the inclusion of the three *Helianthus* hybrid species had almost no effect on the topology of the other species regardless of the data set analyzed. This result is concordant with the observations of McDade (1992), who demonstrated that the inclusion of first-generation hybrids in a phylogenetic analysis of *Aphelandra* had little impact on tree topology unless the hybrids were between distantly related parents. Thus, our results would suggest that hybrid species, like F_1 hybrids, are unlikely to distort phylogenetic relationships among related non-hybrid taxa, and that reticulate evolution should not be considered a major impediment for phylogenetic study of non-hybrid taxa.

4. Conclusions. The data presented here provide testimony to the difficulties associated with the systematic identification of homoploid hybrid derivatives in phylogenetic studies. Although gene tree data will probably be more effective than morphological or chemical data for the detection of hybrid species, many hybrid lineages will be cryptic to this approach as well due to sampling error and the age of the reticulation event. Nonetheless, a large number of hybrid hypotheses have been proposed in the literature based on a variety of biosystematic data sets, and the analytical methods proposed by Morefield (1992) and utilized in this paper provide a powerful tool for evaluating the relative likelihood of these hypotheses.

LITERATURE CITED

Avise, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43: 1192–1208.

Bremer, K. and H.-E. Wanntorp. 1979. Hierarchy and reticulations in systematics. Syst. Zool. 28: 624–627.

Funk, V. 1985. Phylogenetic patterns and hybridization. Ann. Missouri Bot. Gard. 72: 681–715.

Grant, V. 1958. The regulation of recombination in plants. Cold Spring Harbor Symposia on Quantitative Biology 23: 337–363.

_____. 1966a. The origin of a new species of *Gilia* in a hybridization experiment. Genetics 54: 1189–1199.

_____. 1966b. Selection for vigor and fertility in the progeny of a highly sterile species hybrid of *Gilia*. Genetics 53: 757–775.

_____. 1981. Plant Speciation. Columbia Univ. Press, New York.

Heiser, C. B. 1947. Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. Evolution 1: 249–262.

_____. 1949. Study in the evolution of the sunflower species *Helianthus annuus* and *H. bolanderi*. Univ. Calif. Publ. Bot. 23: 157–196.

_____, D. M. Smith, S. Clevenger, and W. C. Martin. 1969. The North American sunflowers (*Helianthus*). Mem. Torrey Bot. Club 22: 1–218.

Hennig, W. 1966. Phylogenetic Systematics. Univ. Illinois Press, Urbana.

Humphries, C. J. 1983. Primary data in hybrid analysis. Pp. 89–103 *in* N. I. Platnick and V. Funk (eds.), Advances in Cladistics: Proceedings of the Second Meeting of the Willi Hennig Society. Columbia Univ. Press, New York.

McDade, L. 1991. Hybrids and phylogenetic systematics I. Patterns of character expression in hybrids and their implications for cladistic analysis. Evolution 44: 1685–1700.

_____. 1992. Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. Evolution 46: 1329–1346.

Morefield, J. D. 1992. Evolution and systematics of *Stylocline* and related Filagininae (Asteraceae: Inuleae). Ph.D. dissertation, Claremont Graduate School, California.

Nelson, G. 1983. Reticulation in cladograms. Pp. 105–111 *in* N. I. Platnick and V. Funk (eds.), Advances in Cladistics: Proceedings of the Second Meeting of the Willi Hennig Society. Columbia Univ. Press, New York.

Rieseberg, L. H. 1991. Homoploid reticulate evolution in Helianthus (Asteraceae): evidence from ribosomal genes. Amer. J. Bot. 78: 1218–1237.

_____, S. Beckstrom-Sternberg, A. Liston, and D. M. Arias. 1991. Phylogenetic and systematic inferences from chloroplast DNA and isozyme variation in *Helianthus* section *Helianthus*. Syst. Bot. 16: 50–76.

_____, R. Carter, and S. Zona. 1990. Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* species (Asteraceae). Evolution 44: 1498–1511.

_____, H. C. Choi, R. Chan, and C. Spore. 1993. Genomic map of a diploid hybrid species. Heredity 70: 285–293.

_____ and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Pl. 5: 65–84.

_____ and J. F. Wendel. 1993. Introgression and its consequences in plants. Pp. 70–109 *in* R. Harrison (ed.), Hybrid Zones and the Evolutionary Process. Oxford Univ. Press, Oxford.

Rogers, C. E., T. E. Thompson, and G. J. Seiler. 1982. Sunflower species of the United States. National Sunflower Association, Bismark, ND.

Rohlf, F. J. 1982. Consensus indices for comparing classifications. Math. Biosci. 59: 131–144.

Schilling, E. E. and C. B. Heiser. 1981. An infrageneric classification of *Helianthus* (Compositae). Taxon 30: 393–403.

Soltis, D. E., P. S. Soltis, and B. G. Milligan. 1992. Intraspecific chloroplast DNA variation: systematic and phylogenetic implications. Pp. 117–150 *in* P. S. Soltis, D. E. Soltis, and J. J. Doyle (eds.), Molecular Systematics of Plants. Chapman and Hall, New York.

Spring, O. and E. E. Schilling. 1989. Chemosystematic investigation of the annual species of *Helianthus* (Asteraceae). Biochem. Syst. Ecol. 17: 519–528.

_____ and _____. 1991. The sesquiterpene lactone chemistry of *Helianthus* sect. *Atrorubentes* (Asteraceae: Heliantheae). Biochem. Syst. Ecol. 19: 59–80.

Swofford, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony, version 3.0. Illinois Natural History Survey, Champaign, IL.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York.

_____. 1957. The hybrid origin of microspecies in the *Elymus glaucus* complex. Cytologia 22S: 336–340.

Wagner, W. H. 1969. The role and taxonomic treatment of hybrids. BioScience 19: 785–789.

_____. 1983. Reticulistics: the recognition of hybrids and their role in cladistics and classification. Pp. 63–79 *in* N. I. Platnick and V. Funk (eds.), Advances in Cladistics: Proceedings of the Second Meeting of the Willi Hennig Society. Columbia Univ. Press, New York.

Watrous, L. and Q. Wheeler. 1981. The outgroup comparison method of character analysis. Syst. Zool. 30: 1–11.

Appendix 1. Morphological characters and character states used for probabilistic tests of hybridity in *Gilia*. Data are recoded from Grant (1966a, 1966b). Intermediate states for multistate characters are not shown.

Multistate	Binary	Character	States 0	States 1
01a	01	central leader short	yes	no
01b	02	central leader erect and long	no	yes
02a	03	lateral branches long	yes	no
02b	04	lateral branches short	no	yes
03	05	orientation of branches	spreading	ascending
04	06	point of departure of lateral branches	arising near plant base	arising in upper part of plant
05a	07	stem thickness < 2 mm	yes	no
05b	08	stem thickness > 3 mm	no	yes
06	09	length of lower leaf lobes	long	short
07a	10	leaf lobes pointing forward	yes	no
07b	11	leaf lobes at right angles to rachis	no	yes
08a	12	inflorescence loose	yes	no
08b	13	inflorescence glomerate	no	yes
09a	14	first flower of a cluster pedicelled	yes	no
09b	15	first flower of a cluster sessile	no	yes
10a	16	corolla limb < 4 mm diam.	yes	no
10b	17	corolla limb > 6 mm. diam.	no	yes
11a	18	corolla pale violet	yes	no
11b	19	corolla deep violet	no	yes
12a	20	sexual maturity < 76 days	yes	no
12b	21	sexual maturity > 87 days	no	yes

Appendix 2. Morphological data set used for probabilistic tests of hybridity in *Gilia*.

Taxon	Binary Characters 5	10	15	20	
G. malior	00000	00000	00000	00000	0
G. modocensis	11111	11011	11111	11111	1
Branch I, F_9	10111	11001	00011	10001	1
Branch II, F_{11}	11111	11111	11010	11101	1
Branch III, F_{10}	11101	11001	01010	00111	0

Appendix 3. Morphological characters and character states used for cladistic analysis of *Helianthus* section *Helianthus*. Data are from Schilling and Heiser (1981), Spring and Schilling (1989), and Rogers et al. (1982). All multistate characters are recoded to additive binary components. Intermediate states for multistate characters are not shown.

			States	
Multistate	Binary	Character	0	1
01a	01	obligate perennial	yes	no
01b	02	obligate annual	no	yes
02a	03	roots fibrous	yes	no
02b	04	tap root	no	yes
03	05	rhizome	absent	present
04	06	stem pubescence absent/sparse	no	yes
05	07	stem green	yes	no
06a	08	stem glabrous	yes	no
06b	09	stem pubescence dense	yes	no
07a	10	leaf hair coverage > 0%	no	yes
07b	11	leaf hair coverage > 50%	no	yes
07c	12	leaf hair coverage < 100%	yes	no
08a	13	plant height < 1 m	yes	no
08b	14	plant height > 2 m	no	yes
09	15	basal rosette	absent	present
10	16	leaf variation along stem	gradual	abrupt
11a	17	leaf arrangement opposite	yes	no
11b	18	leaf arrangement mostly alternate	no	yes
12	19	leaf margin entire	yes	no
13	20	leaf margin revolute	yes	no
14a	21	leaf shape l/w index < 2	yes	no
14b	22	leaf shape l/w index > 5	no	yes
15a	23	leaf base cuneate	yes	no
15b	24	leaf base cordate	no	yes
16	25	capitate glands, lower leaf surface	absent	present
17a	26	leaf hairs short, appressed	yes	no
17b	27	leaf hairs long, slender	no	yes
18a	28	leaf hair base not enlarged	yes	no
18b	29	leaf hair base enlarged, both surfaces	no	yes
19a	30	leaf blade/petiole index < 0.05	yes	no
19b	31	leaf blade/petiole index > 0.20	no	yes
20a	32	ray number < 10	yes	no
20b	33	ray number > 17	no	yes
21	34	ray anthochlor pigments	absent	present
22a	35	ray size < 1.5 cm	yes	no
22b	36	ray size > 2.5 cm	no	yes
23	37	disk flower color	yellow	red
24a	38	disk size < 1.4 cm	yes	no
24b	39	disk size > 2 cm	no	yes
25	40	phyllary number	< 25	25 +
26a	41	phyllaries tightly appressed	yes	no
26b	42	phyllaries reflexed	no	yes

Appendix 3. Continued.

Multistate	Binary	Character	States 0	States 1
27	43	phyllary tip	acute	acuminate
28a	44	phyllary shape index l/w < 3	yes	no
28b	45	phyllary shape index l/w > 5	no	yes
29	46	phyllary basal width	0–4 mm	> 4 mm
30a	47	phyllary hair coverage > 0%	no	yes
30b	48	phyllary hair coverage > 50%	no	yes
30c	49	phyllary hair coverage < 100%	yes	no
31	50	pappus squamellae	usually present	usually absent
32	51	pale length exceeding disk flowers	no	yes
33a	52	pales glabrous	yes	no
33b	53	pale pubescence dense	no	yes
34a	54	pale lobing absent	yes	no
34b	55	pales strongly 3-lobed	no	yes
35	56	style color	yellow	red
36a	57	achenes glabrous	yes	no
36b	58	achene pubescence dense	no	yes
37a	59	achene length < 3 mm	yes	no
37b	60	achene length > 6 mm	no	yes
38	61	self compatibility	yes	no
39	62	stem mottled	no	yes
40	63	capitate glands, anther appendages	absent	present
41	64	capitate glands, upper leaf surface	absent	present
42	65	capitate glands, ligules	absent	present
43a	66	oil content of achenes < 20%	yes	no
43b	67	oil content of achenes > 30%	no	yes
44a	68	linoleic acid content < 50%	yes	no
44b	69	linoleic acid content > 70%	no	yes
45a	70	oleic acid content < 15%	yes	no
45b	71	oleic acid content > 30%	yes	no
46a	72	not resistant to carrot beetles	yes	no
46b	73	very resistant to carrot beetles	no	yes
47a	74	not resistant to aphids	yes	no
47b	75	very resistant to aphids	no	yes
48a	76	not resistant to sunflower beetle larvae	yes	no
48b	77	very resistant to sunflower beetle larvae	no	yes
49a	78	not resistant to leafhoppers	yes	no
49b		very resistant to leafhoppers	no	yes
50	79	branching	reduced	highly branched
51	80	branching mostly above	yes	no
52	81	branching mostly below	no	yes
53	82	habitat dry, sandy	yes	no
54	83	habitat wet	no	yes

Appendix 4. Morphological data set used for cladistic analysis in *Helianthus*. Missing data or polymorphic characters are indicated as "9."

Taxon	Binary Characters																
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	84
section *Agrestes*:																	
Agrestes	11000	00001	00100	01010	10000	00101	01011	01100	10110	00000	01000	00000	11100	99101	01000	11101	0011
section *Divaricati*:																	
atrorubens	00000	01101	10101	10010	10100	00111	11101	01000	00000	00001	00000	11000	00100	99999	91199	11101	0010
maximiliani	00101	01101	00100	01100	00101	00111	01101	10111	10111	01101	01000	00000	00111	10101	01010	11101	0010
porteri	11100	01101	00000	01001	11001	00100	00010	00000	10111	00001	00000	00000	00100	99999	91110	99101	0010
section *Helianthus*:																	
annuus	11100	01101	10100	01110	00111	10001	11111	11111	01100	11101	01011	10010	09110	10101	01100	00001	1010
anomalus	11100	10001	10000	01000	10000	00111	10011	01110	10011	01001	00010	11111	00100	99999	99999	99990	1000
argophyllus	11100	00111	11110	01100	00101	11001	11111	11111	01100	11110	01011	11010	00111	10001	11100	11101	1000
bolanderi	11100	00101	10000	01110	00001	11001	11011	01100	11110	11000	11011	01010	00111	99110	01010	11991	1010
debilis																	
subsp. debilis	10100	00101	00000	01010	00100	00101	11010	01101	10110	01000	01011	11000	00100	11001	19900	99991	1000
subsp. tradiflorus	10100	00001	00000	01010	00109	00101	11010	01101	10110	01000	01011	11000	00109	11111	09999	99991	0000
subsp. vestitus	11100	00111	10000	01010	00109	11101	11010	01101	10110	01100	01011	11000	00109	99999	99999	99991	1000
subsp. *cucumerifolius*	11100	01101	00000	01110	00100	10101	11011	01101	10110	01000	01011	11000	01100	00001	19999	99991	1000
subsp. silvestris	11100	01101	00000	01110	00100	10101	11011	01101	10110	01000	01011	11000	01100	11101	01099	00001	0000
deserticola	11100	01101	10000	01100	10000	10111	11011	01110	10010	01100	01010	11110	00100	99999	90099	99990	1000
exilis	11100	00101	10000	01110	00001	11001	11011	01100	10110	11000	11011	01010	00111	10110	00011	99101	1010
neglectus	11100	00101	00100	01000	00110	00101	11111	11111	10110	01100	11111	10010	00119	10101	01000	99101	1000
niveus																	
subsp. niveus	10110	00111	11000	01000	00001	10001	11010	01100	10010	01111	01110	11110	00111	11101	09999	99991	1100
subsp. canescens	11100	01111	11100	01100	00001	10001	11011	01100	10110	01119	01110	11110	00111	11101	01000	11991	1000
subsp. tephrodes	10110	00111	11000	01000	00001	10001	11011	11100	10010	01111	01110	11110	00111	11101	09900	11101	1100
paradoxus	11100	00001	00100	11000	10000	00111	11011	01100	10010	00000	00010	10000	00000	10101	09900	10001	0011
praecox																	
subsp. praecox	11100	01101	10000	01110	00100	10101	11011	01101	10110	01100	11111	11000	01109	11101	09999	99991	1000
subsp. hirtus	11100	01101	10000	01110	00109	10101	11011	01101	10110	01100	11111	11000	01101	10101	00000	10101	1000
subsp. runyonii	11100	01101	10000	01110	00100	10101	11011	01101	10110	01100	11111	11000	01100	10101	11000	99991	1000
petiolaris																	
subsp. petiolaris	11100	00101	10000	01110	10000	00101	11111	11111	10110	01100	11111	11110	00100	10101	00099	10991	1000
subsp. fallax	11100	01101	00100	01110	10000	00101	11011	01111	10110	01100	11111	11110	00100	11101	09900	99101	1000

Appendix 5. Sesquiterpene lactone data set used for cladistic analysis in *Helianthus*. Data are from Spring and Schilling (1989, 1991). The compound with retention time of 0.38 (Spring and Schilling, 1989) was not scored because it was not present in significant amounts in any of the annual sunflowers. Missing data or polymorphic compounds are indicated by the number nine. A compound was scored as present only if it occurred in significant quantities.

	Binary Characters									
Taxon	5	10	15	20	25	30	35	40	45	50
section *Agrestes*:										
agrestes	90000	09001	00000	00000	00000	00000	00009	09019	00009	09090	00
section *Divaricati*:										
atrorubens	10009	00909	90099	09001	00000	00000	00090	00000	00000	00000	00
maximiliani	09909	00001	00900	90009	00000	00009	00090	00000	00000	00090	00
porteri	91099	00011	00910	00009	00000	00000	09000	09900	00000	00000	00
section *Helianthus*:										
annuus	00000	00090	10001	00001	01001	00000	01000	00001	90000	00000	00
anomalus	00100	00090	00100	00009	00009	00000	00090	00009	00000	00100	90
argophyllus	00000	00090	90001	09009	00001	01009	01091	00009	00000	00000	00
bolanderi	00000	00090	10009	00009	00001	01009	00000	00001	11100	00100	00
debilis										
subsp. *debilis*	00100	00100	01000	10101	00000	00010	00000	00000	00001	00000	00
subsp. *tardiflorus*	00100	00100	01000	10101	00000	00010	00000	00000	00001	00000	00
subsp. *vestitus*	00100	00100	01000	10109	00000	00000	00000	00000	00001	00000	00
subsp. *cucumerifolius*	00100	00100	01000	10101	00000	00010	00000	00000	00001	00001	00
deserticola	00000	00000	10100	00000	00009	09000	01011	00000	00010	00000	00
neglectus	00900	00090	10000	00001	09000	00000	00000	00900	10000	00190	00
niveus										
subsp. *niveus*	00000	00000	10009	00010	10010	10100	10100	00901	00000	00000	01
subsp. *canescens*	00000	00000	00009	00001	00009	00900	00100	00009	10000	00900	00
subsp. *tephrodes*	00000	00000	10009	01001	00001	00900	00000	00101	10000	00100	00
paradoxus										
praecox										
subsp. *praecox*	00000	00100	01000	10901	00101	09100	00000	90000	10091	00190	00
subsp. *hirtus*	00000	00100	01000	10101	00101	01901	00000	90000	00091	00190	00
subsp. *runyonii*	00000	00110	10000	10001	00101	00100	00900	00001	00001	10190	00
petiolaris										
subsp. *petiolaris*	00000	90900	00000	10001	00000	00000	00000	00000	00001	00000	00
subsp. *fallax*	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00

Appendix 6. Chloroplast and nuclear ribosomal DNA data set used for cladistic analysis in *Helianthus*. Characters 1–32 are nuclear rDNA restriction site mutations 1–32 from Rieseberg (1991, Table 3). Characters 33–46 are rDNA length mutations recoded in binary form from characters 33–41 in Rieseberg (1991, Table 3). Characters 47–94 are cpDNA restriction site mutations from Rieseberg et al. (1991, Table 6) and are as follows: 47 = *Apa* I, 48–51 = *Ava* I, 53–54 = *Bam*H I, 55–58 = *Ban* I, 59–60 = *Bcl* I, 61–64 = *Bst*N I, 65–66 = *Bst*X I, 67–70 = *Cla* I, 71–74 = *Dra* I, 75–76 = *Eco*R I, 77 = *Hinc* II, 78–79 = *Hind* III, 80 = *Nco* I, 81–83 = *Nde* I, 84 = *Nhe* I, 85 = *Pst* I, 86 = *Sac* I, 87 = *Sna*B I, 88–89 = *Spe* I, 90 = *Stu* I, 91–92 = *Xba* I, and 93–94 = *Xmn* I.

	Binary Characters																		
Taxon	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	94
section *Divaricati*:																			
atrorubens	11000	10001	00000	01011	00000	10010	00000	00000	00000	00000	00010	00000	00100	00010	00000	00001	00011	01000	0000
maximiliani	11000	00000	00000	01000	00010	10010	00000	00000	00001	00000	00000	00000	00100	00010	00000	10001	01011	00000	0100
porteri	11010	00001	00000	00000	00010	11001	00000	00000	00101	00000	00000	00000	00000	00100	00000	00000	00000	00010	1100
section *Helianthus*:																			
annuus	00000	00001	00100	01001	01010	01010	10000	01100	00111	10000	10000	00000	10000	10000	00100	00000	00000	00001	0000
anomalus	10100	00001	00101	11101	01110	11110	11011	01100	00101	00000	10000	00000	11000	10000	00100	00100	00000	00101	0000
argophyllus	00000	00001	00100	01001	01010	01010	10100	01100	00111	10000	10000	10000	10000	10000	00100	00000	00000	00000	0000
bolanderi	10000	00001	00000	00001	00010	00010	00000	01100	00111	10000	10000	00000	10000	10001	10100	00110	00000	10100	0000
debilis																			
subsp. *cucumerifolius*	10100	00001	10010	00101	11110	11110	01000	00010	00101	00000	11000	00001	10010	10000	00100	00100	00000	00000	0000
subsp. *debilis*	10101	00001	10010	00101	11100	11110	01000	00010	00101	00000	10000	00110	10000	10000	00100	00000	10000	00000	0000
subsp. *silvestris*	10100	01001	10010	00101	11110	11110	01000	00010	00101	00100	10000	00000	11000	11000	01100	00100	00000	00100	0000
subsp. *tardiflorus*	10100	00001	10010	00101	11100	11110	01000	00010	00101	00000	10000	00010	10000	10000	00100	00000	10000	00000	0000
subsp. *vestitus*	10100	00101	10010	00101	11100	11110	01000	00010	00101	00000	10000	00010	10000	10000	00100	00000	10000	00000	0000
deserticola	10100	00011	00101	11101	01110	11110	11011	01101	10111	10010	10000	00000	11000	10000	00100	00100	00100	00100	0000
exilis	10000	00001	00000	00001	00010	01010	00000	01100	00111	11001	10100	00000	10000	10000	10111	00100	00000	00100	0000
neglectus	10100	00000	00001	10101	01110	11010	01011	00000	00101	00000	10000	00000	11000	10000	00100	00100	00100	00100	0000
niveus																			
subsp. canescens	10100	00001	00001	10101	01110	11010	01011	00000	00101	00000	10001	00000	11000	10000	00100	00100	00000	00100	0000
subsp. niveus	10110	00001	01000	11101	01110	11010	01000	00000	00101	00000	10000	00000	10000	10000	00100	00000	00000	00000	0000
subsp. tephrodes	10110	00001	00001	10101	01111	11010	01010	00000	01001	00000	10000	00000	11000	10000	00100	00100	00000	00100	0000
paradoxus	10100	00001	00101	11101	01110	11110	11011	11100	01111	10000	10000	00000	10000	10000	00100	00000	00000	00001	0000
petiolaris																			
subsp. fallax	10100	00000	00001	10101	01110	11110	01011	00000	00101	00000	10000	00000	11000	10000	00100	00100	00100	00100	0000
subsp. petiolaris	10100	00000	00001	10101	01110	11110	01011	00001	00101	00000	10000	00000	11000	10000	00100	00100	00000	00100	0000
praecox																			
subsp. hirtus	10100	00001	10011	00101	11110	11110	01000	00010	00101	00000	10000	00000	10000	10000	00100	00100	00000	00000	0000
subsp. praecox	10100	00001	10011	00101	11110	11110	01000	00010	00101	00000	10000	00000	10000	10000	00100	01000	00000	00000	0000
subsp. runyonii	10100	00001	10011	00101	11110	11110	01000	00010	00101	00000	10000	00000	10000	10000	00100	00000	00000	00000	0000

THE EVOLUTION OF SELF-INCOMPATIBILITY IN FLOWERING PLANTS: A PHYLOGENETIC APPROACH[1]

Stephen G. Weller[2], Michael J. Donoghue[3], and Deborah Charlesworth[4]

ABSTRACT. A phylogenetic approach was used to address three questions: (1) Was self-incompatibility (SI) ancestral in angiosperms? (2) Did gametophytic and sporophytic self-incompatibility arise independently? and (3) Is SI in heteromorphic species homologous with SI in homomorphic species? Parsimony analyses using several available hypotheses of phylogenetic relationships and different codings of the incompatibility character imply that self-compatibility (SC) was probably ancestral in angiosperms. It does not appear that the evolution of SI was a major factor in the evolutionary radiation of flowering plants. Sporophytic SI appears to have evolved independently in each family where it is known to occur, and in most cases SC, rather than gametophytic SI, appears to have been the precursor condition. Heteromorphic SI has clearly evolved on numerous occasions, probably from SC precursors, and it is unlikely that heteromorphic SI has evolved from homomorphic, sporophytic SI through loss of alleles. Although phylogenetic approaches provide some valuable insights on the evolution of SI, it is clear that phylogenetic relationships are still poorly understood, as is the distribution of self-incompatibility. Furthermore, detailed genetic information on SI is available for only a small number of species. Additional information from both areas is needed to answer the questions we have outlined more definitively.

A resurgence of interest in phylogeny reconstruction has resulted in the application of phylogenetic analysis to a diversity of evolutionary questions, some of which have previously been addressed primarily by population biologists (e.g., Donoghue, 1989; Brooks and McLennan, 1991; Armbruster, 1992; McDade, 1992; Norton et al., 1993). Our purpose is to examine several questions related to the evolution of self-incompatibility using a phylogenetic approach, as suggested earlier by Olmstead (1989). These questions are: (1) Was self-incompatibility ancestral in angiosperms? (2) Did gametophytic and sporophytic self-incompatibility arise independently, or is there evidence that one system evolved from the other? and (3) Is self-incompatibility in heteromorphic species homologous with self-incompatibility in homomorphic species? The questions raised here are similar to those asked by Charlesworth (1985); the major difference is our more explicit phylogenetic approach.

For our first question, the ancestral condition in angiosperms, it was possible to perform a preliminary phylogenetic analysis, taking into account ambiguity in estimates of phylogeny and in the taxonomic distribution of compatibility systems. Our treatment of the second and third questions is less detailed, mainly because appropriate phylogenies are not yet available.

[1]We are grateful to Dick Olmstead, Spencer Barrett, and Susanne Renner for helpful discussion of these issues. Sean Graham, Shirley Graham, Doug Soltis, and Pam Soltis kindly made available unpublished phylogenetic information. We thank Lucinda McDade, Todd Stuessy, Shirley Tucker, and Del Wiens for their unpublished observations on breeding systems. Peter Hoch and an anonymous reviewer provided helpful comments on the manuscript.

[2]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A.
[3]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, U.S.A.
[4]Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, U.S.A.

SELF-INCOMPATIBILITY SYSTEMS

In hermaphroditic plant species, self-compatibility (SC) occurs when pollen grains produced by an individual germinate and effect fertilization following self-pollination. Self-incompatibility (SI) represents a complex and widespread collection of breeding systems (de Nettancourt, 1977). Among the flowering plants, SI systems result in rejection of self pollen or outcross pollen possessing the same incompatibility type. How this reaction takes place is variable, and a number of classification schemes are possible based on the genetics underlying SI and variation in associated morphological features. Perhaps the most basic distinction is between homomorphic and heteromorphic SI (de Nettancourt, 1977). In homomorphic systems, the flowers of all incompatibility groups are morphologically similar. Such systems are widely distributed, occurring in as many as 60–90 angiosperm families (Charlesworth, 1985; Gibbs, 1988). The number of incompatibility types in a population may be large, numbering in the dozens. Genetic control of homomorphic systems is variable. In species with gametophytic self-incompatibility (GSI), the incompatibility reaction of the pollen grain is determined by its own genotype (de Nettancourt, 1977). Identity between the allele occurring in the pollen grain and one of the alleles in the pistil is sufficient to prevent fertilization. The number of loci controlling GSI is variable, ranging from 1 to 4, although the number of cases with more than a single locus is small (Lundqvist, 1975). Sporophytic self-incompatibility (SSI) involves determination of the pollen incompatibility reaction by the parental sporophyte (de Nettancourt, 1977). All pollen grains produced by a plant have the same incompatibility reaction, even though they may have different genotypes. Identity between the reaction expressed by the pollen grain and the stigma reaction is sufficient to result in rejection. Nearly all studies have shown that SSI systems are governed by a single locus (de Nettancourt, 1977).

Brewbaker (1957, 1967) demonstrated that in most species GSI is correlated with binucleate pollen, stylar inhibition, wet stigmas, and long-term viability of stored pollen. In contrast, species with SSI usually have trinucleate pollen, stigmatic inhibition, dry stigmas, and short-term viability of stored pollen. Among the 23 flowering plant families with single-locus SI in which crosses have been used to test for the occurrence of GSI versus SSI, there are only two exceptions to the correlation of SSI and trinucleate pollen and GSI and binucleate pollen (Table 1). Pollen cytology (presence of binucleate vs. trinucleate pollen) may thus be useful for predicting the probable genetic basis of self-incompatibility when other information is lacking.

In heteromorphic systems, found in 25–27 plant families, two or three incompatibility groups occur in populations, each type having a different arrangement of stamens and styles in the flowers (Darwin, 1877). Differences among the morphs are reciprocal, such that stigmas and anthers at the same level occur in different morphs. Pollinations between anthers and stigmas at the same level are generally the only ones leading to production of seeds. In dimorphic systems, incompatibility is controlled by a single locus with two alleles. In trimorphic systems, two loci, each with two alleles, control expression of incompatibility types in most cases (Barrett, 1992). Determination of incompatibility types is sporophytic in all cases. As in homomorphic SI, heteromorphic species also show a correlation between pollen cytology and site of pollen tube inhibitions (Brewbaker, 1957).

GENERAL METHODS

Detection of self-incompatibility is often difficult, as emphasized by Charlesworth (1985). The most thorough studies involve a complete genetic analysis for the determination of gametophytic versus sporophytic SI. Such studies are rare, and almost nonexistent for woody plants with long generation intervals. In the absence of such studies, crossing pro-

grams involving selfs and outcrosses can be very useful, particularly if genetically distinct individuals can be identified with certainty. Results from such crosses are not always clear-cut, chiefly because it may be difficult to rule out inbreeding depression as the cause of low seed production after self-pollination, unless seed production is near zero (Charlesworth, 1985; Krebs and Hancock, 1990). Studies that rely on observations of low fruit set of isolated plants are generally not adequate for assessment of SI, nor are observations of low fruit set in natural populations. Low pollinator activity may be the cause of reduced seed or fruit production under such conditions. Observations of pollen tube growth can help distinguish between pre-zygotic SI and other causes of low seed set, and the site of inhibition of pollen tube growth provides suggestive information on the occurrence of GSI versus SSI.

Table 1. Pollen cytology (presence of binucleate vs. trinucleate pollen), site of inhibition, and genetic systems controlling homomorphic self-incompatibility. **Boldface** indicates exceptions to association of sporophytic SI and trinucleate pollen for cases of single-locus SI. References provided in Charlesworth (1985).

Family or Genus	Site of Inhibition (Where Known)	SI System	Pollen Cytology
Asteraceae	stigma	S	3
Betulaceae	**stigma**	**S**	**2**
(*Corylus*)			
Brassicaceae	stigma	S	3
Bromeliaceae	--	G	2
(*Ananas*)			
Caryophyllaceae	stigma	S	3
Chenopodiaceae	--	G (multi-locus)	3
(*Beta*)			
Commelinaceae	stigma	G	2
Convolvulaceae	stigma	S	3 (2 in other species)
(*Ipomoea leucantha*)			
Cornaceae	stylar	G	2
Poaceae	stylar	G (2 locus)	3
Fabaceae			
Mimosoideae	stylar	G	2 (3 in *Calliandra*)
Caesalpinoideae	--	--	2
Papilionoideae	stylar	G	2
Liliaceae	stylar	G	2
Nyctaginaceae	stylar	S	3
Onagraceae	stigma	G	2
Papaveraceae	stigma	G	2
Ranunculaceae	--	G (3–4 loci)	2
Rosaceae	style	G	2
Rubiaceae	stigma	--	2
(*Coffea*)		S	3
(*Galium*)	--	G	2
Saxifragaceae	style	G	2
Scrophulariaceae	style	G (single or multi-locus)	2
Solanaceae	style		
Sterculiaceae	**ovary**	S	2
Theaceae	--	G	2

The reports of SI used in this study are from a database assembled by Charlesworth (1985), augmented with more recent studies. Because the adequacy of the studies varies greatly, we have been conservative in our approach, accepting SI as present in a species only when crosses have been carried out. In a few cases we have used the ancillary features of SI, including site of pollen inhibition and stigma type as an indicator of probable gametophytic versus sporophytic control. Characterization of the type of SI in a family may be based on one or a few reports. Broader sampling within a family has usually revealed identical mechanisms controlling SI (Charlesworth, 1985), although Lewis (1977) has reported multi-locus SSI in the Brassicaceae, where single-locus SI is far more common. Assumptions of uniform genetic mechanisms in a family based on limited sampling represent a possible source of error.

The phylogenies referred to in this study are based on morphological and/or molecular evidence. In cases where the degree of phylogenetic information is limited, we consider alternative hypotheses from a phylogenetic perspective, but have not been able to trace character changes onto trees. In addressing the question of the ancestral condition in angiosperms we were able to use MacClade (vers. 3.0, Maddison and Maddison, 1992), to determine the minimum number of changes along the branches of a particular tree that are needed to account for the distribution of the states of a character found at the tips of the branches (in the terminal taxa). This parsimony optimization (or mapping) procedure establishes the states present at the internal (or ancestral) nodes in the tree and hence the branches along which changes of state are assumed to have occurred.

In order to account for uncertainties about phylogenetic relationships we have considered a variety of different phylogenetic hypotheses. In view of uncertainties concerning the breeding systems present in some taxa, we have also explored the robustness of our conclusions using a range of character codings. Furthermore, we have explored the parsimony of alternate hypotheses of character evolution by "fixing" the state at particular nodes using the "paintbrush" tool in MacClade. This allows us to calculate (and compare) the minimum number of steps that are needed to account for character evolution under the assumption that a particular state was present at given nodes in the tree.

THE ORIGIN OF SELF-INCOMPATIBILITY IN FLOWERING PLANTS

Historical background. In 1950, Whitehouse stated that "...the abrupt rise of the angiosperms from the gymnosperms may have been the result of...the increased efficiency of sexual reproduction made possible by multiple-allelomorph incompatibility." In his view, SI is a retained, ancestral trait (symplesiomorphy) in angiosperms, and self-compatibility (SC) derived. If Whitehouse is correct, then all systems of SI would be homologous.

Crowe (1964) also suggested that incompatibility systems in angiosperms were inherited from more ancient ancestors. She presented a "phylogenetic arrangement" of outbreeding systems in the angiosperms (Figure 1), showing GSI as ancestral and SSI derived from it. In this scheme, heteromorphic systems are suggested to have evolved from homomorphic SSI through loss of alleles.

More recently other authors have suggested the early evolution of SI in angiosperms and the direct derivation of divergent SI systems. Lundqvist (1975) proposed that the presence of complex multi-locus GSI systems in Ranunculaceae and Chenopodiaceae is consistent with a close phylogenetic relationship between these families, as well as a connection to monocots with similar systems (grasses). De Nettancourt (1977) viewed the scattered occurrence of incompatibility systems in the flowering plants as evidence that SI was ancestral. On the basis of pollen wall sculpturing of fossil pollen, Zavada (1984) concluded that the origin of SSI coincided with the appearance of the earliest recognizable angiosperm pollen.

Bernhardt and Thien (1987) suggested that GSI was present in the "protoangiosperms," based on the supposed widespread occurrence of SI in primitive angiosperms.

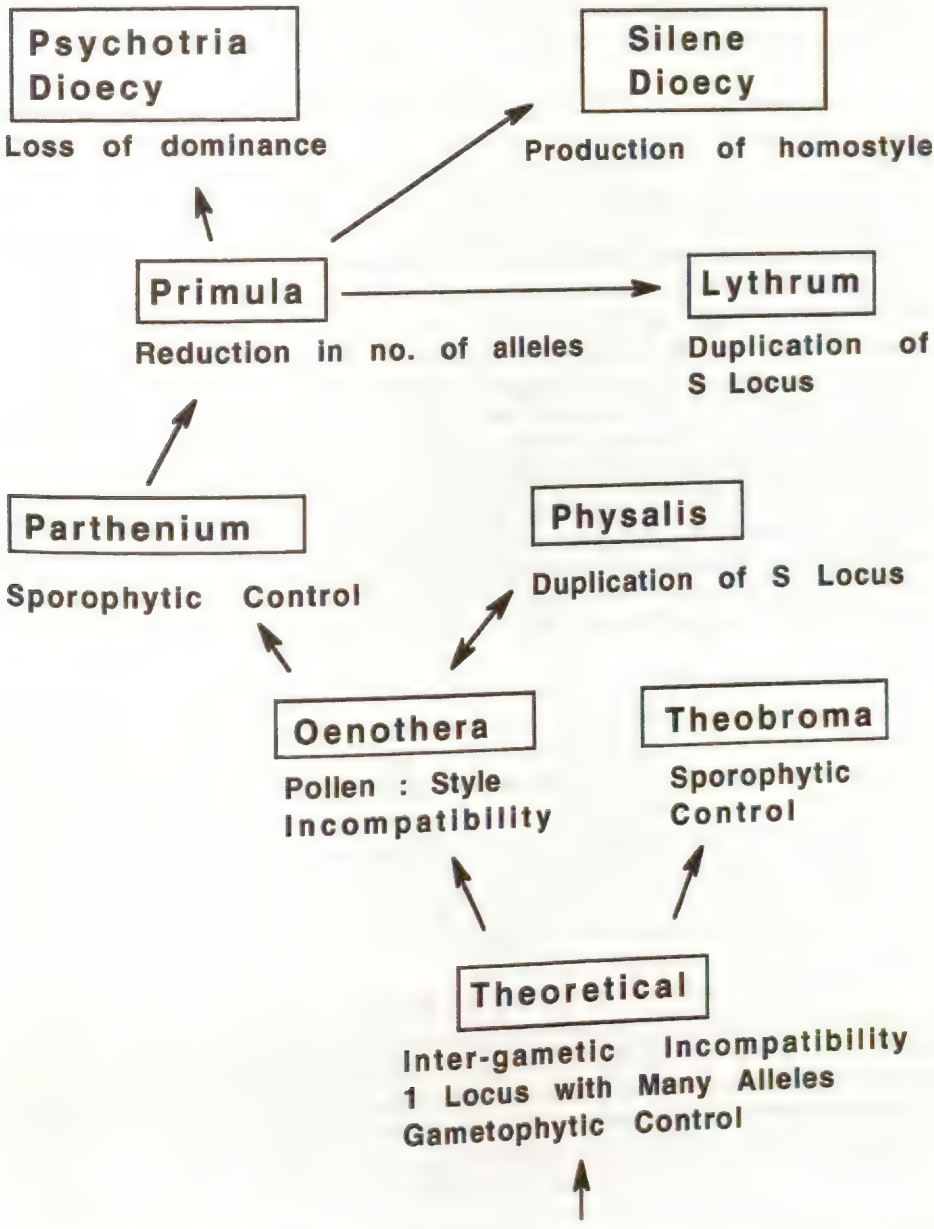


Figure 1. Relationships of self-incompatibility (SI) systems in angiosperms, based on Crowe (1964). Gametophytic self-incompatibility (GSI) is shown as primitive and sporophytic self-incompatibility (SSI) derived from it. Heteromorphic systems are suggested to have evolved from homomorphic SSI through loss of alleles. In this "phylogenetic" arrangement, the first angiosperms were believed to have possessed SI.

In contrast, Bateman (1952) stated that "The highly dispersed incidence of self-incompatibility throughout the Angiosperms is an indication that it has arisen *de novo* a number of times." He further predicted that because they have evolved on many occasions, SI systems should be highly variable. While not rejecting the possibility that SC could be derived, Bateman pointed out that SC could be primitive in many groups. Charlesworth (1985) and Olmstead (1989) suggested the multiple origin of SI on the basis of uniform SI systems within families compared to the variety of systems found among families.

Taxa and trees used in the analysis. In the analyses presented below, we consider a number of alternative phylogenetic hypotheses involving the 27 terminal taxa used by Donoghue and Doyle (1989) in their analysis of basal relationships in angiosperms (see Figure 2). These taxa were selected by Donoghue and Doyle (1989) to represent all potential early lines of angiosperm evolution. In particular, they included many families of Magnoliidae (sensu Cronquist, 1988; see Stebbins, 1974; Takhtajan, 1991), some including only a single genus (and therefore represented by a generic name in Figure 2), as well as some "placeholders," a term used for presumably monophyletic lineages containing several to many families. When enough information was available for the placeholder taxa, we have assigned a code reflecting the inferred ancestral condition in the group, rather than using single exemplar taxa, which might be nested well within the group, and therefore be less likely to represent the ancestral condition. Thus, all monocots were represented by a single taxon in the analysis, on the assumption that these plants form a monophyletic group within angiosperms (i.e., that the root of the angiosperms is not within monocots). Likewise, Monimiaceae(+) represents the so-called "core Laurales," including Hernandiaceae and Lauraceae; Ranunculaceae(+) represents ranunculids, including Berberidaceae, Menispermaceae, Lardizabalaceae, and Papaveraceae; and Hamamelidales(+) represents all of the tricolpate dicot groups (eudicots; Doyle and Hotton, 1991), including rosids, dilleniids, caryophyliids, and asterids.

In view of uncertainties still surrounding the phylogenetic relationships of these taxa, we made use of a number of the alternate trees found by Donoghue and Doyle (1989), as well as trees representing the results of ribosomal RNA sequences (Hamby and Zimmer, 1992), sequences of the chloroplast gene *rbc*L (Chase et al., 1993), and other morphological data sets (Loconte and Stevenson, 1991). For purposes of comparison, the taxa used in all trees are those included in Donoghue and Doyle (1989). To produce trees that could be compared, taxa used in Donoghue and Doyle were rearranged to simulate trees from later studies. Owing to variation in the taxa included in the different analyses, it was not possible to represent exactly trees from the later studies. We were able, however, to capture the basic features of alternative hypotheses for angiosperm evolution.

Although the taxa delimited by Donoghue and Doyle (1989) may be appropriate for examining relationships among major lines of angiosperms, they are not ideal from the standpoint of examining the evolution of SI, because in some taxa (especially larger ones) both SC and SI are known to occur (Table 2). In cases where there was insufficient information to determine whether the basal condition was SC or SI, we coded the taxon as polymorphic (SC/SI). When there are only two character states this amounts to coding the taxon as "unknown." In a few cases discussed below we felt we could safely assign a single code (SC or SI) to a polymorphic taxon based on a previous analysis of phylogenetic relationships within the group in question, which we could use in order to assess which condition was ancestral in the group based on parsimony. Finally, in view of these uncertainties and the possible problems associated with polymorphic terminal taxa (Nixon and Davis, 1991), we also explored the robustness of the results to a variety of alternative codings described below. A

detailed phylogenetic analysis of each of the more inclusive groups in the study would be useful, but beyond the scope of this paper. The use of different coding systems, as well as the analysis of various phylogenetic hypotheses, helps in circumventing the problems associated with inclusion of large placeholders.

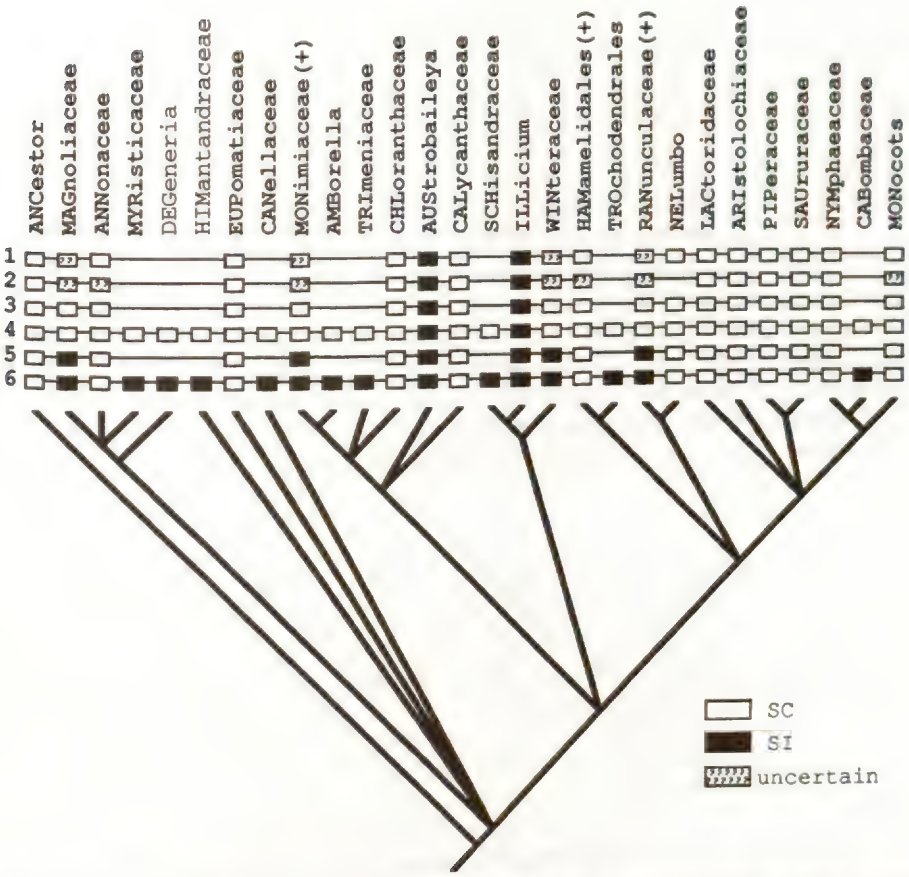


Figure 2. Consensus tree from Donoghue and Doyle (1989) showing the 27 angiosperm taxa and the six character codings discussed in the text. Open boxes are self-compatible (SC), black boxes are self-incompatible (SI), and patterned boxes are polymorphic (SC/SI); the absence of a box represents lack of information or questionable evidence (coded as unknown). Codings 1–6 are used to encompass uncertainties in interpretation of breeding system data. Coding 1 is our preferred coding, based on our assessment of reports in the literature (in coding 1, open boxes are used to represent several groups known to possess SI [Annonaceae, Hamamelidales(+), monocots] because phylogenetic information suggests that SC is basal in each group). In coding 2, the three polymorphic taxa (Annonaceae, Hamamelidales(+), and monocots) are recoded as polymorphic. In coding 3, all polymorphic taxa are recoded as SC, and in coding 4, both polymorphic and unknown taxa are coded as SC. Codings 5 and 6 favor SI: in coding 5, all polymorphic taxa are recoded as SI, and in coding 6, both polymorphic and unknown taxa are recoded as SI. Each of the six character codings was optimized on each of the most parsimonious trees based on Donoghue and Doyle (1989)—not on the consensus—and on a variety of alternative trees (see text and Figure 4), using MacClade. See text for full discussion.

Table 2. Distribution of breeding systems and pollen cytology of taxa used in phylogenetic analysis of early angiosperm lines (Figures 3–6). Pollen cytology is based on Brewbaker (1967). Taxa are listed in order shown in Figure 3. In several polymorphic taxa (Annonaceae, Hamamelidales(+), monocots) references indicating basal SC are listed. For Hamamelidales (+) and monocots, only basal families and their breeding systems are listed; other reports for SC and SI are not listed. Dashes indicate lack of information.

Taxon	Families or Subclasses (for inclusive taxa)	Breeding System	Pollen Cytology	References for Breeding System Information
Magnoliaceae	--	SC/SI	2	Heiser, 1962; McDaniel, 1963; Gottsberger, 1977; Parks et al., 1983
Annonaceae	--	SC/SI (coded as SC)	2	Bawa, 1974; Vithanage, 1984; SC basal: J. Doyle, pers. comm.
Myristicaceae	--	--	2	
Degeneria	--	--	2	Thien, 1980
Himantandraceae	--	--	--	
Eupomatiaceae	--	SC	--	Endress, 1984
Canellaceae	--	--	2	
Monimaceae(+)	Hernandiaceae	--	2	
	Lauraceae	SC/SI	2	Free, 1970; Kubitzki and Kurz, 1984; Wheelwright, 1985;
	Monimiaceae	SC/SI	2	Lorence, 1985
Amborella	--	--	--	
Trimeniaceae	--	--	--	
Chloranthaceae	--	SC	2	Endress, 1984
Austrobaileya	--	SI	--	Prakash and Alexander, 1984
Calycanthaceae	--	SC	2	L. McDade, pers. comm.
Schisandraceae	--	--	2	
Illicium	--	SI	2	Thien et al., 1983
Winteraceae	--	SC/SI	2	Gottsberger et al., 1980; Godley and Smith, 1981; Lloyd and Wells, 1992
Hamamelidales(+)	Rosidae Dilleniidae Caryophyllidae Asteridae	SC/SI (coded as SC)	2 and 3	Numerous reports of SC and SI, SC basal based on phylogenies of Hufford and Crane, 1989
Trochodendrales	Trochodendraceae	--	2	
Ranunculaceae(+)	Berberidaceae Menispermaceae Lardizabalaceae Papaveraceae Ranunculaceae	SC/SI	2	East, 1940; Fryxell, 1957; Moore, 1968; Humphreys and Gale, 1974; Østerbye, 1977; Lawrence, 1975; Rust and Roth, 1981; Suzuki, 1983
Nelumbo	--	SC	--	Sohmer, 1977
Lactoridaceae	--	SC	--	T. Stuessy, pers. comm.; D. Wiens, pers. comm.
Aristolochiaceae	--	SC	2	Wildman, 1950; Tanaka and Yahara, 1989; M. Donoghue, pers. obser.
Piperaceae	--	SC	2	Semple, 1974
Saururaceae	--	SC	2	S. Tucker, pers. comm.
Nymphaeaceae	--	SC	2	Prance and Anderson, 1976; Schneider and Moore, 1977; Ervik et al., in press
Cabombaceae	--	--	--	Schneider and Jeter, 1982
Monocots	--	SC/SI (coded as SC)	2 and 3	Numerous reports of SC and SI, SC basal based on phylogenies of Dahlgren and Bremer, 1985; G. Bharathan, pers. comm.

Coding of taxa. Reliable data are not available for every group (Table 2). Studies of breeding systems of magnoliid taxa have been difficult because most are woody plants, often found in remote areas, which makes crossing programs impractical. A number of these species also seem to have very low fecundity, regardless of the type of pollination, and this complicates the interpretation of experimental efforts to verify the occurrence of SC versus SI. The absence of a report of SI cannot be equated with SC, and may simply mean that the breeding system has not been investigated. There is also a tendency not to report negative results, in this case the presence of SC.

The best documented cases of SI occur in *Illicium floridanum* (Illiciaceae, Thien et al., 1983), and *Austrobaileya scandens* (Austrobaileyaceae, Prakash and Alexander, 1984). Only a single individual of *Austrobaileya* was used in crosses, although observations of pollen tubes indicated a failure of growth typical of SI (Prakash and Alexander, 1984). We have coded *Illiciaceae* and *Austrobaileya* as SI in the experiments described below (Table 2).

Several other reports of SI seem well founded, but there are also SC species within these terminal taxa and the ancestral condition in the group is uncertain. For this reason, we have coded Monimiaceae(+), Winteraceae, Magnoliaceae, and Ranunculaceae(+) as polymorphic (SC/SI; Table 2). These are discussed in turn below.

With respect to Monimiaceae(+), reports of SI include four genera of Lauraceae (Kubitzki and Kurz, 1984), and several species of *Tambourissa* (Monimiaceae, Lorence, 1985). Bernhardt and Thien (1987) cited *Ocotea* (Lauraceae) as likely to be SI on the basis of a report of a single isolated tree that failed to set fruit (Wheelwright, 1985). *Persea* (Lauraceae), described as SI by Free (1970) and Kubitzki and Kurz (1984), was found to be SC by Wheelwright (1985). Interpretation of data for Lauraceae is complicated by the pronounced synchronized heterodichogamy characteristic of this family, which makes it difficult to carry out self or geitonogamous pollinations because the stigmas are rarely receptive when the anthers are shedding pollen.

In Winteraceae, *Pseudowintera colorata* has been reported as SI (Godley and Smith, 1981; Lloyd and Wells, 1992). In "chase pollination" experiments in *Pseudowintera colorata*, the application of self pollen to stigmas 1–3 days prior to outcrossed pollen resulted in significant reductions in seed production (Lloyd and Wells, 1992), indicating the possibility of SC and ovule abortion due to inbreeding depression. For this reason, and because SC is also well documented in Winteraceae (Gottsberger et al., 1980), this family is coded as SI/SC in our preferred coding. *Belliolum*, *Tasmannia*, and *Zygogynum* (Thien, 1980), and *Exospermum* (Bernhardt and Thien, 1987), are not accepted as SI according to our criteria.

The claim of SI in the Magnoliaceae (McDaniel, 1963) is based primarily on failure of seed production by a few isolated trees of several *Magnolia* spp. grown outside their range. It is difficult to rule out lack of pollinators among other potential factors that could explain these results. Because we lack phylogenetic information within Magnoliaceae we have coded this taxon as SC/SI, a conservative approach given the methods used to detect SI and the predominance of SC in the family (Gottsberger, 1977; Heiser, 1962; McDaniel, 1963, Parks et al., 1983).

SI is well documented within Ranunculaceae and related taxa, but because of uncertainties about phylogenetic relationships within ranunculids they are also coded as SC/SI. *Nelumbo* is coded as SC on the basis of Snigirevskaya's (1964) observations indicating facultative self-pollination, and Sohmer's (1977) crossing study.

In a few cases where SI and SC occur within the same taxon we felt more confident in assessing the ancestral breeding system condition, and these taxa are coded as SC in our preferred coding (see below). Annonaceae is coded as SC because the only report of SI is for *Sapranthus* (Bawa, 1974), which is well nested within the family (J. A. Doyle, pers. comm.).

All other investigated Annonaceae are reported to be SC. Similarly, despite the widespread occurrence of SI in both "placeholder" taxa, SC appears to be basal in the Hamamelidales(+) and related taxa, based on phylogenies of Hufford and Crane (1989), and in the monocots, based on phylogenies of Dahlgren and Bremer (1985). This also appears to hold in the phylogenies of Chase et al. (1993). The monocots were considered polymorphic or SC in various runs, but never SI, because there is no phylogenetic information suggesting that the basal monocots are SI. As information on the phylogeny and breeding systems of placeholder taxa accumulates, these groups can be analyzed in more detail.

Despite possible biases against publishing accounts of SC, there is good evidence for the widespread occurrence of SC among "primitive" angiosperms, especially among paleoherbs (Donoghue and Doyle, 1989; Table 2). In the Aristolochiaceae, two species of *Asarum* (Wildman, 1950; Tanaka and Yahara, 1989) are known to be SC, and *Saruma* also appears to be SC (Donoghue, unpubl.). SI also seems to be absent in Lactoridaceae (D. Wiens and T. Stuessy, pers. comm.), Saururaceae (S. Tucker, pers. comm.), Piperaceae (Semple, 1974), and Nymphaeaceae (Prance and Anderson, 1976). Although Bernhardt and Thien cite Schneider and Moore (1977) as evidence for SI in *Nuphar*, our interpretation of their data suggests little support for SI. Recent studies by Ervik et al. (in press) indicate experimental evidence for self-compatibility in *Nuphar*. Our assessment of SC in the Lactoridaceae and Saururaceae is based on observations of abundant fruit set on isolated individuals. In those magnoliids which have not already been discussed, there is evidence for SC in Eupomatiaceae (Endress, 1984) and Calycanthaceae (L. McDade, pers. comm.; S. Weller, unpubl. obser.). The Chloranthaceae also appear to be SC, although data are restricted to a single species in the family (Endress, 1987).

We coded as "unknown" those taxa for which we lack any information, and those for which we find the available information too difficult to interpret. Information on breeding systems is missing for a number of critical taxa including *Amborella*, Canellaceae, Himantandraceae, Myristicaceae, Schisandraceae, Trimeniaceae, and Trochodendrales. Widespread occurrence of dioecy complicates assessment of incompatibility systems in Myristicaceae and Schisandraceae. Other problematical cases include *Degeneria* (Degeneriaceae), and *Cabomba* (Cabombaceae). Although cited as SI by Bernhardt and Thien (1987), in these cases there is insufficient evidence in the original report to confirm the presence of SI. For example, Schneider and Jeter (1982) used bagging studies to confirm only that pollination is required for seed production in *Cabomba*, and did not attempt to self-pollinate bagged flowers. In Degeneriaceae no information on SI was presented in the original report (Thien, 1980).

Finally, based on outgroup comparison (Maddison et al., 1984), we coded the hypothetical ancestor used to root the tree by Donoghue and Doyle as SC (although coding it as unknown yielded the same basic results discussed below). Gnetales are considered more closely related to angiosperms than are conifers, cycads, or *Ginkgo* (e.g., Doyle and Donoghue, 1986; 1993), but these groups are dioecious, and it is therefore difficult to establish whether they would be (or once were) SC or SI. Although it is possible that an extinct hermaphroditic group more closely related to angiosperms than Gnetales may have been SI, this breeding system is unknown among non-angiosperm seed plants. In contrast, SC has been well documented in conifers, the only extant "gymnosperms" with cosexual species (Sarvas, 1962; Franklin, 1970; Sorensen, 1982). The evolution of SI may be associated with the occurrence of the style in flowering plants.

The result of these deliberations is our preferred coding of the terminal taxa, which we will call coding number 1 (Table 2, Figure 2). In view of the uncertainties considered above, however, and in recognition that some of our decisions might be wrong, we also considered a set of alternative codings (2–6 in Figure 2). In coding 2, the three polymorphic taxa

coded as SC in coding 1 (Annonaceae, Hamamelidales(+), and monocots; Table 2) were recoded as polymorphic. Codings 3 through 6 explore the effect of coding polymorphic and unknown taxa as either SC or SI. Codings 3–6 bracket the possibilities by favoring either SC (codings 3 and 4) or SI (codings 5 and 6). In codings 3 and 5 the taxa coded as SC/SI in coding 1 are coded as SC and SI, respectively. Codings 4 and 6 are even more extreme, in that positive codes are assigned both to the polymorphic taxa in coding 1 and to the taxa coded as unknown. In coding 4 all uncertainties are resolved as SC, while in coding 6 all uncertainties are coded as SI.

Analyses. Using the codings described above, breeding systems were optimized on different phylogenetic trees using three approaches. Under the first hypothesis, no assumptions were made regarding the presence of SI versus SC at the base of the angiosperms. Under the second hypothesis, SI was assumed to have been present at the base of the angiosperms, but could also evolve later from SC ancestors. In the final hypothesis, it was assumed that SI was basal in the angiosperms, and that all cases of SI in terminal taxa are homologous, representing retention of this breeding system from SI ancestors.

Optimizing our preferred coding of breeding systems (coding 1, Figure 2) on all 54 of the most parsimonious trees of Donoghue and Doyle (1989), we obtained the basic results in Figure 3, which shows only one of the most parsimonious trees of Donoghue and Doyle. It is always most parsimonious to assign SC to the base of the angiosperms, which then entails a minimum of six independent origins of SI (Figure 3A; note that four of the origins are at the base of, or within, the polymorphic terminal taxa, and that these are required in every calculation). Assigning SI to the base of the angiosperms entails three extra steps involving loss of SI (Figure 3B). Fixing SI at the base of angiosperms, however, does not ensure that all instances of SI are homologous, or retained from the common ancestor in which it evolved. For example, the occurrence of SI in Magnoliaceae and in Ranunculaceae(+) (Figure 3B) must be seen as separate originations. Insuring that all coded instances of SI are potentially retained from a single origin at the base of angiosperms generally requires an additional six steps (Figure 3C). Based on these analyses, it is more parsimonious to suppose that SC is ancestral in angiosperms and that SI originated several times early in angiosperm evolution.

To account for uncertainty about phylogenetic relationships we optimized character coding 1 on a series of other phylogenetic trees (Figure 4). Using a slightly less parsimonious tree (one additional step is required) from Donoghue and Doyle (1989), which roots the tree among paleoherbs, it is again most parsimonious to assume that SC is ancestral. An additional seven steps are required to ensure that SI is retained from a single origin (Figure 4A). When the taxa used by Donoghue and Doyle (1989) are rearranged to simulate the Hamby and Zimmer (1992) rDNA tree, the root is again among paleoherbs (Figure 4B). As before, SC is ancestral, but nine extra steps are required to make SI a retained condition. When the Donoghue and Doyle taxa are arranged to match the *rbc*L sequence tree of Chase et al. (1993), the tree is rooted between a monosulcate clade and a tricolpate clade (Figure 4C). In this case only five steps are required to place SC at the base of the angiosperms (with SI homologous in *Austrobaileya* and Illiciaceae), while seven extra steps are required if SI is homologous and retained from a common ancestor. The tree presented by Loconte and Stevenson (1991), based on morphological characters, yields the same basic conclusion.

With only a few exceptions (see below), each of the alternative codings discussed above yielded results suggesting that it is most parsimonious to assume that SC is ancestral in angiosperms and that SI evolved several times independently. In the case of codings 2–4 the number of steps separating "SC basal" from "SI basal" and from "SI retained" is generally the same as described above for coding 1. As expected, the number of steps separating these hypotheses is somewhat reduced under codings 5 and 6. In all cases coding 5 favors SC at

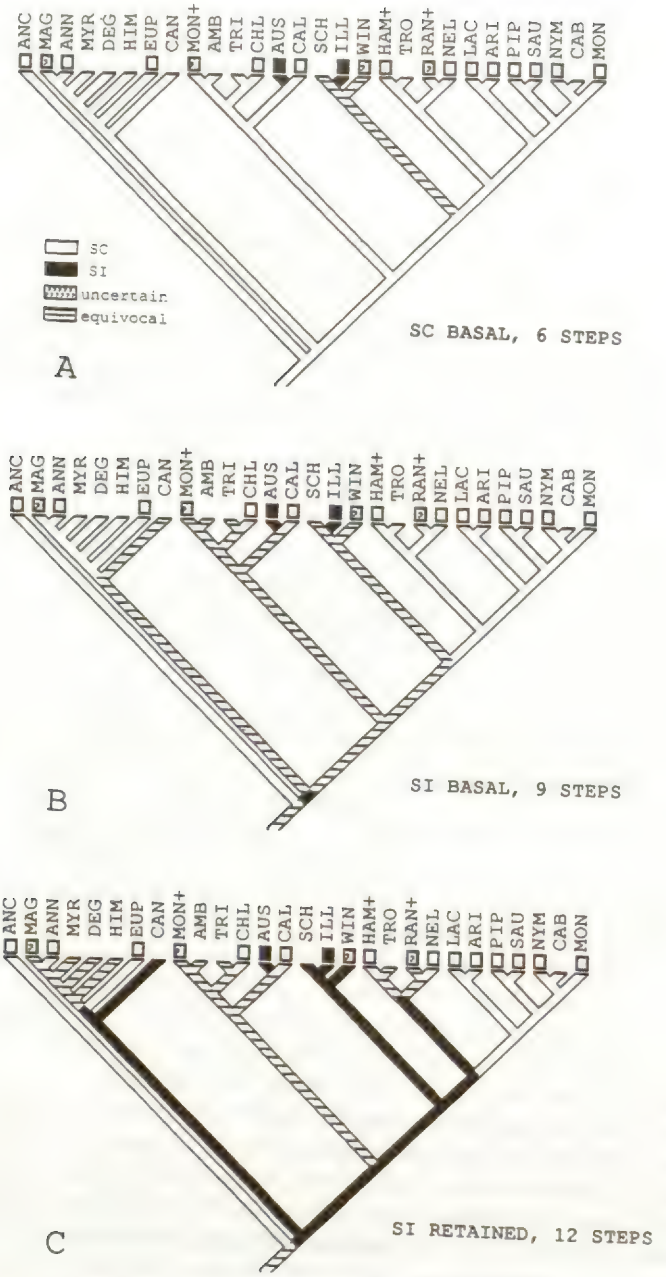


Figure 3. Optimizations of character coding 1 (see Figure 2) on the "preferred" most parsimonious tree of Donoghue and Doyle (1989), using MacClade; abbreviations are the first three letters of the taxon names in Figure 1. In MacClade the states at the tips of the branches and at the internal nodes are indicated by different shading, and a change from one state to another is marked by a change of shading. In some cases it is equally parsimonious to assign either state to a position on a tree, and such instances are indicated by diagonal lines. **A**. Most parsimonious hypothesis; SC ancestral in angiosperms. **B**. SI "fixed" at the base of angiosperms (using the "paintbrush" tool in MacClade); some instances of SI are not retained from this origin. **C**. Most parsimonious optimization, allowing all coded instances of SI to be retained from the common ancestor of angiosperms.

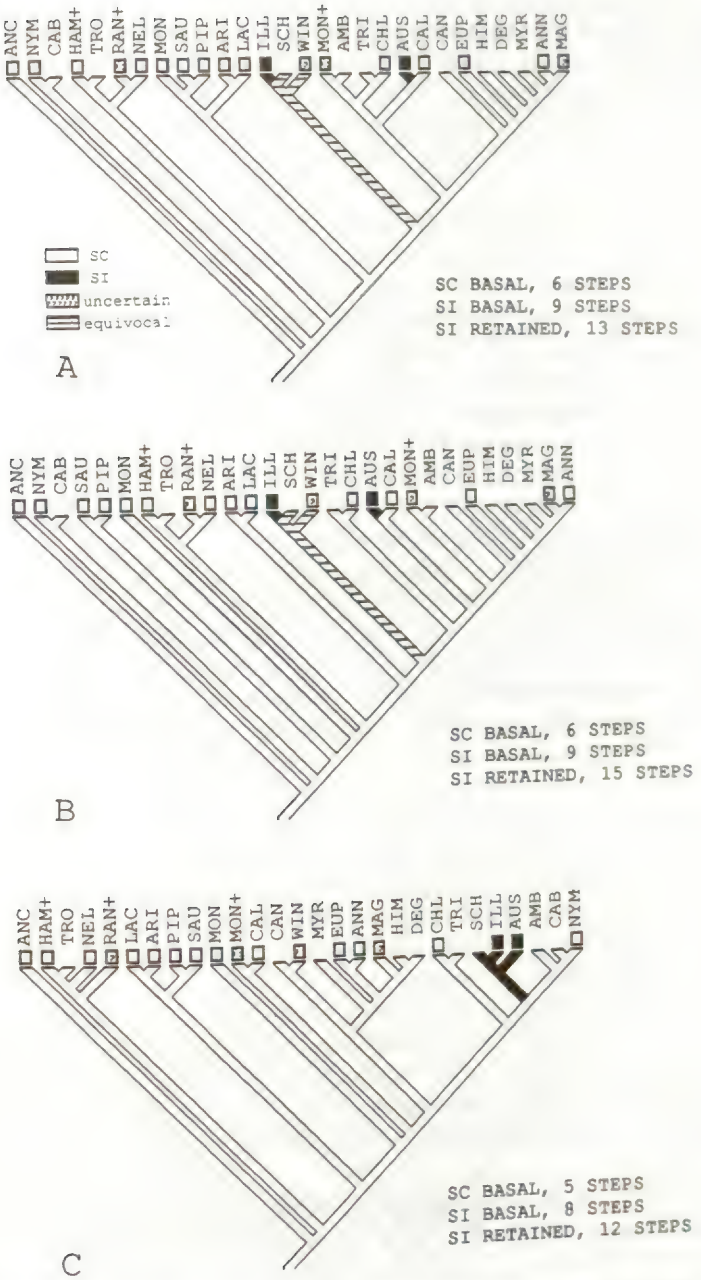


Figure 4. Optimization of character coding 1 (see Figure 2) on alternative phylogenies employing MacClade; SC is ancestral in all cases. Only the most parsimonious optimizations (SC basal in each case) are shown. For purposes of comparison, the taxa used in all trees are those included in Donoghue and Doyle (1989); these taxa were rearranged to simulate trees from later studies. Owing to variation in the taxa included in the different analyses, it was not possible to represent exactly trees from the later studies; see text for details. **A**. Donoghue and Doyle's (1989) "Nymphaeales-basal" tree. **B**. Tree representing Hamby and Zimmer's (1992) results based on ribosomal RNA sequences. **C**. Tree representing Chase et al.'s (1993) results based on sequences of the chloroplast gene *rbc*L.

the base of the angiosperms. Surprisingly, even coding 6, which was designed to strongly favor ancestral SI, slightly favors SC at the base of most of the trees we considered. Several of Donoghue and Doyle's morphological trees, however, yield SI basal with coding 6 (Figure 5A). In these cases, however, trees with SC basal require only one additional step (Figure 5B). Even under these circumstances, it is more parsimonious to assume that SC is ancestral than it is to assume that SI has been retained in all cases from the first angiosperm (Figure 5C).

Conclusions. If transitions between SI and SC are equally weighted, we conclude from these analyses that it is more parsimonious to suppose that SC is ancestral in angiosperms than it is to assume (1) that SI is ancestral, with the possibility of SI also evolving later from SC ancestors, or (2) that SI was ancestral, and all taxa coded as having SI evolved from ancestors also possessing SI. This result holds under a wide variety of codings and for all recent phylogenetic hypotheses. Except under coding 6, the difference in number of steps ranges from 5 to 9. It therefore seems unlikely that SI was a major factor in the success and radiation of angiosperms, as suggested by Whitehouse (1950) and Crowe (1964). If the first angiosperms were colonizing species (Stebbins, 1974; Bond, 1989; Taylor and Hickey, 1992; Doyle and Donoghue, 1993), the occurrence of SC in these species would be consistent with the pattern found in extant colonizing species (Baker, 1965).

Results based on equal weighting of SC and SI imply that transitions between the two breeding systems are similar in both directions, which seems improbable given the evidence for repeated origin of SC from SI within some angiosperm families (e.g., Asteraceae, Brassicaceae). These observations led Stebbins (1957) to suggest that it is easier to lose than to gain SI. In our analyses, weighting against the independent gain of SI would tend to favor placing SI at the base of the angiosperms. How could one determine the appropriate weighting scheme? Although the probability of losing vs. gaining SI is not known, and may vary in different angiosperm lineages, one future approach might be to investigate a variety of weighting schemes, and test the threshold weighting value that would favor placing SI at the base of the angiosperms. If the analysis suggested that only a low probability of SI to SC transitions would prevent placing SI at the base of the angiosperms, the differences in the number of steps found in this study should be interpreted with caution. Despite these caveats, it seems clear based on other evidence that SI systems have evolved independently elsewhere in the angiosperms (see below).

Our conclusion that SC is basal in angiosperms could also be overturned if (1) phylogenetic trees with Magnoliales in the basal position are best supported (this seems unlikely in view of recent molecular data, which tend to support paleoherb rootings); (2) all, or most, of the taxa coded as SC/SI and as unknown are actually SI; (3) SI is discovered among angiosperm outgroups; or (4) extinction of basal lineages possessing SI has occurred.

THE DERIVATION OF SPOROPHYTIC SELF-INCOMPATIBILITY

Few flowering plant families, all of them tricolpate dicots (Donoghue and Doyle, 1989), possess SSI. This form of SI has been claimed on the basis of genetic information in 8 families: Asteraceae, Betulaceae, Brassicaceae, Caryophyllaceae, Convolvulaceae, Nyctaginaceae, Rubiaceae, and Sterculiaceae (Lundqvist, 1990). Our attention will focus on these families. An additional 4 families—Cactaceae (Ganders, 1976), Caprifoliaceae (East, 1940; Pojar, 1974), Lobeliaceae (East, 1940), and Polygalaceae (Zapata and Arroyo, 1978; Sobrevila and Arroyo, 1982)—have SI, verified through crossing programs, and these may be SSI based on their possession of trinucleate pollen. Homomorphic multi-allelic incompatibility

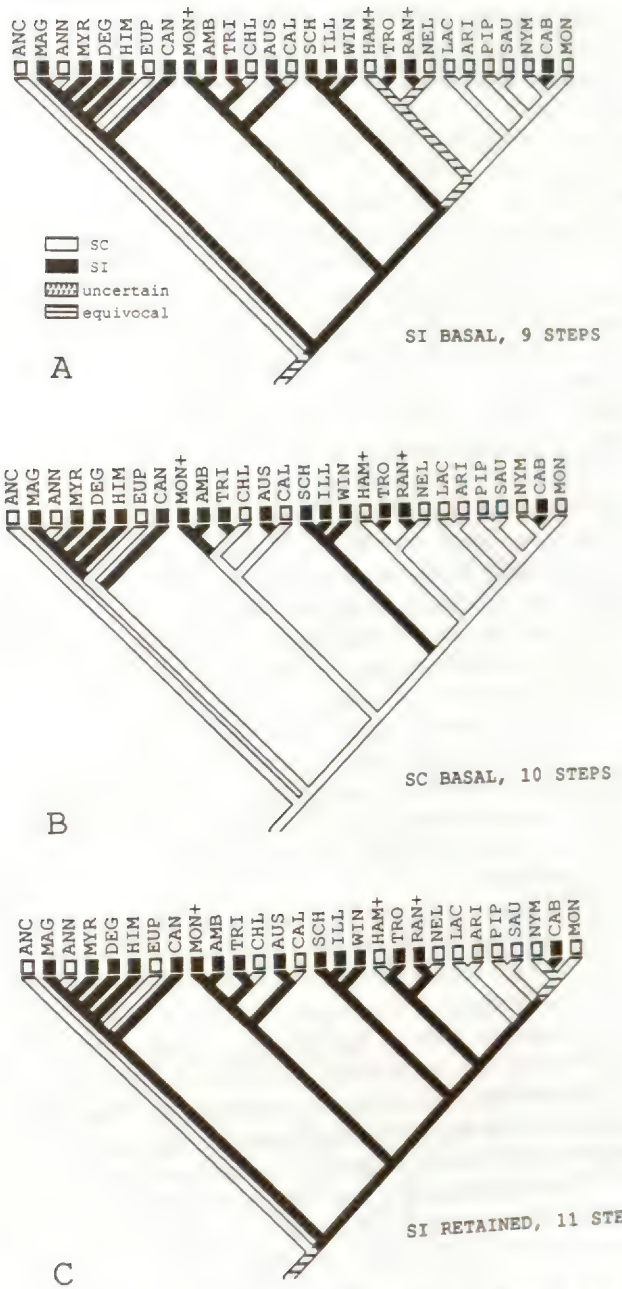


Figure 5. Optimizations of character coding 6 (see Figure 2) on the "preferred" most parsimonious tree of Donoghue and Doyle (1989), using MacClade. **A**. Most parsimonious hypothesis; SI ancestral in angiosperms. **B**. An optimization with SC ancestral. **C**. Most parsimonious optimization allowing all coded instances of SI to be retained from the common ancestor of angiosperms.

is claimed for some Boraginaceae (Dulberger, 1970; Crowe, 1971; Schou and Philipp, 1983), a family typically characterized by SC or heteromorphic incompatibility, although inbreeding depression seems as likely a reason for self-sterility in *Borago officinalis*.

The occurrence of SSI among the more specialized angiosperm families has long been viewed as evidence for the derived nature of this form of SI (Brewbaker, 1957; Crowe, 1964; Brewbaker, 1967). Here we consider two questions from a phylogenetic perspective: first, did SSI evolve independently in the families where it occurs, and second, did SSI evolve from GSI, or from SC?

For those cases with genetic data supporting the occurrence of SSI, as well as those where SSI is likely, it appears that SSI evolved independently in each family where it occurs. Cronquist's (1988) view that different families possessing SSI are not directly related has been supported by morphological and molecular cladistic analyses (e.g., Hufford, 1992; Olmstead et al., 1992; Chase et al., 1993). The likely independent evolution of SSI suggests that the underlying molecular mechanisms could well be different in each case. Unfortunately, only a single family with SSI, Brassicaceae (Nasrallah and Nasrallah, 1986; Haring et al. 1990), has been investigated. Molecular studies of Asteraceae, or other families with SSI, would be very valuable.

What genetic systems preceded the evolution of SSI? Sporophytic self-incompatibility is probably the ancestral condition in Asteraceae, based on the widespread distribution of this form of incompatibility in the different tribes. Phylogenetic analyses of Asteridae (sensu Cronquist, 1988) and Asterales (Olmstead et al., 1992; Olmstead et al., 1993) provide little evidence for precursor conditions to SSI in Asteraceae, again because there is insufficient information on breeding systems for key families (Table 3). Although phylogenetic studies of Asteraceae have provided a good idea of relationships among the major lines (Bremer et al., 1992), we still lack information on the breeding systems of crucial basal groups. Several species of Mutisieae are known to possess SI (Carlquist, 1974; Kalin Arroyo, pers. comm.), although no tests have been carried out to determine the type of SI. Information on breeding systems in the Barnedesinae would be especially useful.

Too little is known of the details of SI in Betulaceae for application of phylogenetic analysis. Aside from *Corylus*, for which detailed crossing studies have demonstrated SSI (Thompson, 1979), self-incompatibility is known in *Alnus* and *Betula* (Hagman, 1975), but no details of genetic control are available. There is no information on breeding systems of related families (Crane, 1989).

All SI species of Brassicaceae that have been investigated have shown sporophytic control, but as in Asteraceae, the breeding systems of the basal, perhaps less-derived genera (e.g., *Stanleya*) have not been investigated (Table 3). The breeding systems of the genera of Capparaceae most closely related to Brassicaceae (Judd et al., 1994; Chase et al., 1993), including *Cleome* and *Polanisia*, have not been studied in detail, although *Cleome spinosa* is SC (Stout, 1923). Based on this limited information (Table 3), SSI probably evolved after the divergence of the Brassicaceae, and the precursor state may have been SC.

In the Caryophyllaceae the only known SI species is *Cerastium arvense* (Lundqvist, 1990). SC is widespread in the family, suggesting that SSI in *Cerastium* evolved independently from SC in the Caryophyllaceae. In related families (e.g., Rodman et al., 1984) SC is the only reported breeding system for hermaphroditic species, further indicating the *de novo* origination of SSI within the Caryophyllaceae. Elsewhere in the Caryophyllidae, SSI is found only in the Nyctaginaceae, where it has been documented in *Bougainvillea* (Zadoo and Khoshoo, 1975). The uniform occurrence of trinucleate pollen in the Nyctaginaceae may indicate sporophytic control where SI is found. The Nyctaginaceae may have evolved within the Phytolaccaceae, where only SC and dioecy have been documented.

Table 3. Breeding systems and pollen cytology (2 vs. 3 nucleate pollen), where known, in relatives of families (either related families or taxa within the family in question) where SSI is claimed. Pollen cytology is taken from Brewbaker (1967). Pollen cytology is given for the family unless there is variation within the family, in which case information is noted for the lowest taxonomic level listed by Brewbaker. SI is homomorphic except where noted. Closely related families with no information are listed to highlight need for more information on these critical families.

Families Possessing SSI and Selected Reports Providing Genetic Evidence	Phylogenetically Related Taxa	Breeding System	Pollen Cytology	References
Asteraceae	Calyceraceae		2	
Parthenium argentatum (Gerstel, 1950)	*Moschopsis insulata*	partially SI		Arroyo and Squeo, 1990
Crepis foetida (Hughes and Babcock, 1959)	Lobeliaceae			
Cosmos bipinnatus (Crowe, 1954)	*Isotoma axillaris*	SI?	3	East, 1940
	Goodeniaceae		2	
	Leschenaultia tubiflora	SI	--	Darwin, 1878
	Sellieria radicans	SI	--	Schonland (in Engler & Prantl, cited by East, 1940)
	Menyanthaceae		2?	
	Villarsia spp.	homomorphic and heteromorphic SI	--	Ornduff, 1988
Betulaceae	Fagaceae		2	
Corylus avellana (Thompson, 1979)	*Castanea* spp.	SI	--	Hagman, 1975
Brassicaceae	Capparaceae		2	
Iberis amara (Bateman, 1955)	*Capparis pitteri*	SI	--	Bawa et al., 1985
	C. verrucosa	SI	--	Bullock, 1985
	Flacourtiaceae		2	
	Casearia (3 spp.)	SI	--	Bawa et al., 1985; Bullock, 1985
	Moringaceae	no information		
	Tovariaceae	no information		
Caryophyllaceae	Phytolaccaceae		3	
Cerastium arvense (Lunqvist, 1990)	*Phytolacca decandra*	SC	--	East, 1940
	Rivinia spp.	SC	--	East, 1940
Convolvulaceae	Solanaceae (many spp.)	GSI	2	East and Manglesdorf, 1925
Ipomoea batatas (Joseph and George, 1983)				
Nyctaginaceae	Phytolaccaceae (see above)			
Bougainvillea (Zadoo and Khoshoo, 1975)				
Rubiaceae	Rubiaceae			
Galium (Crowe, 1964)	Cinchoneae			
	Exostema caribaeum	SI	--	Bullock, 1985
	Coussareae			
	Coussarea (2 spp.)	heteromorphic SI	--	Bawa and Beach, 1983
	Faramea (2 spp.)	heteromorphic SI	--	Bawa and Beach, 1983
	Rubieae	SI	3	
	Gardenieae		2	
	Gardenia thunbergii	SI	--	East, 1940

Table 3. Continued.

Families Possessing SSI and Selected Reports Providing Genetic Evidence	Phylogenetically Related Taxa	Breeding System	Pollen Cytology	References
	Hamelieae			
	Hamelia patens	SI	--	Bawa and Beach, 1983
	H. versicolor	SC	--	Bullock, 1985
	H. xerocarpa	SI	--	Bawa and Beach, 1983
	Posoqueria grandiflora	SI	--	Bawa and Beach, 1983
	Isertieae		3	
	Mussaenda luteoloa	SI	--	East, 1940
	Psychotrieae			
	Cephaelis elata	heteromorphic SI	--	Bawa and Beach, 1983
	Palicourea angustifolia	heteromorphic SI	--	Sobrevila and Arroyo, 1982
	P. petiolaris	SC	--	Sobrevila and Arroyo, 1982
	Psychotria (3 spp.)	heteromorphic SI	--	Bawa and Beach, 1983
	Psychotria (2 spp.)	SC	--	Sobrevila and Arroyo, 1982; Bawa and Beach, 1983
	Rudgea cornifolia	heteromorphic SI	--	Bawa and Beach, 1983
	R. karstenii	SC	--	Sobrevila and Arroyo, 1982
Sterculiaceae *Cola nitida* (Jacob, 1980)	Sterculiaceae		2	
	Ayenia micrantha	SI	--	Bullock, 1985
	Guazuma tomentosa	SI	--	Zapata and Arroyo, 1978
	G. ulmifolia	SI	--	Bullock, 1985
	Physodium corybosum	SI	--	Bullock, 1985
	Sterculia chicha	SI	--	Taroda and Gibbs, 1982
	Melochia sp.	heteromorphic SI	--	Ganders, 1979
	Waltheria sp.	heteromorphic SI	--	Ganders, 1979
	Bombacaceae		2	
	Pachira insignis	SI	--	East, 1940
	Elaeocarpaceae		2	
	Muntingia	SC	--	Bawa and Webb, 1983
	Malvaceae		2	
	Abutilon spp.	SI	--	East, 1940; Sears, 1937
	A. hybridum	SI	--	East, 1940
	Hibiscus syriacus	SI	--	East, 1940
	Tiliaceae		2	
	Goethalsia meiantha	SI	--	Bawa et. al., 1985
	Luhea speciosa	SI	--	Bawa et. al., 1985

In Convolvulaceae, SSI has been documented in *Ipomoea* (Kowyama et al., 1980; Joseph and George, 1983), and is likely in *Argyreia* (Gupta et al., 1981), which is SI and has stigmatic inhibition of pollen tubes. In possibly related families (see Olmstead et al., 1992, 1993), the array of SI systems is complex, and depending on their exact relationships to Convolvulaceae, SSI could have evolved in the common ancestor of several families.

SSI was claimed by Crowe (1964) for *Galium mollugo* (Rubiaceae), although no crossing data were presented to support the claim. The occurrence of trinucleate pollen

in *Galium* is consistent with the presence of SSI (Brewbaker, 1967). Elsewhere in the genus SC and dioecy are known and in the family as a whole homomorphic SI and heterostyly are known (Table 3). The evolutionary relationships among breeding systems in Rubiaceae are unclear. Recent phylogenetic studies based on cpDNA data (Bremer and Jansen, 1991; Bremer and Struwe, 1992), while promising, are still not detailed enough to settle these issues.

Breeding systems in Sterculiaceae appear to be as complex as those in the Rubiaceae (Table 3). Both heteromorphic and homomorphic SI are known (Charlesworth, 1985). Crossing studies using *Cola nitida* indicate that control is sporophytic (Jacob, 1980); in *Theobroma* SSI has been reported, although it is complex (Knight and Rogers, 1955). Because a range of SI systems is present in the Sterculiaceae, and the phylogenetic relationships within the family are so poorly understood, little can be said about precursor conditions to SSI. GSI is likely in related families (Table 3).

To summarize, there is little evidence that GSI preceded SSI in the eight families where SSI is known with certainty, with the possible exception of the Sterculiaceae. If SSI is present in *Galium*, a change from GSI to SSI may have occurred in the Rubiaceae. In Nyctaginaceae, Caryophyllaceae, Betulaceae, and Convolvulaceae, the more likely precursor condition appears to be SC, while in Asteraceae and Brassicaceae information is too preliminary to make an assessment.

THE RELATIONSHIP OF HOMOMORPHIC AND HETEROMORPHIC INCOMPATIBILITY

Phylogenetic analyses (e.g., Chase et al., 1993) support earlier arguments that heteromorphic SI has evolved a number of times (Ganders, 1979; Charlesworth, 1985; Gibbs, 1988; Lloyd and Webb, 1992). In monocots, it occurs in only three families, Amaryllidaceae, Pontederiaceae, and Iridaceae; each of these probably represents a separate origin. Among dicots, heteromorphic SI appears to have evolved independently in every family where it is found (Table 4), with the possible exceptions of Gentianaceae (possibly nested within Loganiaceae) and Santalaceae (perhaps within Olacaceae). This is a minimum number of originations, as heterostyly appears to have evolved several times within some families (see below). Crowe (1964), and later Muenchow (1981) argued that the S locus is homologous in heteromorphic and homomorphic species. Most hypotheses for the evolution of heteromorphic SI suggest a separate origin for heteromorphic and homomorphic systems (Charlesworth, 1982).

Studies of the mechanism of SI in heterostylous species indicate that the underlying controls are different for homomorphic and heteromorphic SI (Stevens and Murray, 1982; Gibbs, 1986; Scribailo and Barrett, 1991; Barrett and Cruzan, 1994). Oppositional systems (incompatibility triggered by like-by-like protein recognition), which are likely to provide the basis for homomorphic SI, seem unlikely to account for incompatibility in tristylous species. In tristylous species, pollen grains from the two sets of anthers produced by a single individual have different incompatibility reactions, leading Charlesworth (1979) and Lloyd and Webb (1992) to argue that developmental factors probably control the different incompatibility reactions rather than incompatibility proteins.

Can phylogenetic methods be applied to this question? Charlesworth (1985) noted that if loss of SI alleles accounted for the evolution of heterostyly from homomorphic ancestors possessing SSI, one might expect the two breeding systems to occur in the same families. There was little evidence for this in her study (Charlesworth, 1985). Here we ask whether in families where both homomorphic SI and heteromorphic SI occur there is any phylogenetic evidence that homomorphic SI directly preceded heteromorphic SI, and if so, whether control of SI was gametophytic or sporophytic.

Table 4. The occurrence of homomorphic self-incompatibility (SI) in families with heteromorphic SI. For families that may possess both heteromorphic and homomorphic SI, cases are listed separately where evidence for presence of heterostyly or homomorphic SI is weak. See text for details.

Families with only heteromorphic SI:	Clusiaceae, Connaraceae, Erythroxylaceae, Gentianaceae, Linaceae, Loganiaceae, Lythraceae, Oleaceae, Oxalidaceae, Plumbaginaceae, Pontederiaceae, Santalaceae, Turneraceae
Families possibly with both heteromorphic and homomorphic SI:	
Strong evidence for both heterostyly and homomorphic SI:	Acanthaceae, Amaryllidaceae, Iridaceae, Menyanthaceae, Olacaceae, Rubiaceae, Saxifragaceae, Sterculiaceae
Weak evidence for heterostyly:	Ericaceae, Fabaceae, Polemoniaceae
Weak evidence for homomorphic SI:	Boraginaceae, Primulaceae

Among families possessing heterostyly, 13 may contain species with homomorphic GSI or SSI, though several of these are weakly documented (Table 4). This list of joint occurrences includes Amaryllidaceae, where tristyly has been widely recognized only recently (Lloyd et al., 1990), Fabaceae, where the occurrence of distyly in *Bauhinia* is in doubt (Lloyd et al., 1990), and the Ericaceae and Polemoniaceae, because of recent reports indicating the possibility of heterostyly (Barrett, 1992). The list does not include Lythraceae, where the claim for homomorphic SI in *Cuphea* (East, 1940) is no longer accepted (S. Graham, pers. comm.). In most cases of joint occurrence, GSI is the most likely mechanism underlying incompatibility in the homomorphic species. Possible exceptions to this generalization are in Boraginaceae and Sterculiaceae, where either trinucleate pollen or genetic data (respectively) suggest the possibility of SSI.

A simple listing of families with heteromorphic and homomorphic SI does not reflect the size of the group in which there is a joint occurrence, the frequency of each breeding system within a family, nor phylogenetic relationships within each clade. As noted by Baker (1984), different breeding systems may have evolved independently in large, diverse families. For example, in the Iridaceae heterostyly occurs in *Nivenia* (Ornduff, 1974), a woody genus that is not likely to bear any close phylogenetic relationship with genera possessing homomorphic SI (*Freesia*, *Gladiolus* [East, 1940]). A report of heterostyly in *Geissorhiza* (Ornduff, 1974) may indicate a closer phylogenetic relationship between heterostylous and homostylous SI, although the nature of the SI system, if any, has not been demonstrated in this genus.

SI is widespread in the Amaryllidaceae, suggesting that tristylous *Narcissus* species may have evolved from an ancestor with homomorphic SI. In *Villarsia* (Menyanthaceae), homomorphic and heteromorphic SI as well as SC are known (Ornduff, 1988). Ornduff suggested that heterostyly may have evolved from a homomorphic SI ancestor, but a phylogenetic analysis of these species is needed to evaluate this hypothesis. Homomorphic and heteromorphic SI are also known in the Olacaceae (Tomlinson, 1974; Zapata and Arroyo, 1978) and possibly the Primulaceae (East, 1940; Pojar, 1974; Ganders, 1979), although again

phylogenetic relationships are uncertain. The pattern of variation in the Rubiaceae is especially complex, although there is some evidence that different types of SI may be partitioned among the tribes. For example, homomorphic SI is found in the Hamelieae (East, 1940; Bawa and Beach, 1983; Bullock, 1985), whereas heterostyly is common in the Psychotrieae (Table 3; Bawa and Beach, 1983). A single genus in the Saxifragaceae (*Jepsonia*) is characterized by heteromorphic SI (Ganders, 1979), whereas some other members of the family have homomorphic SI (Arasu, 1970; Gornall and Bohm, 1984). More information on SI in *Telesonix*, the apparent sister group of *Jepsonia* (Soltis et al., 1993), would help in understanding the origin of distyly in this genus.

Homomorphic SI and heterostyly may occur together in the Boraginaceae, where several monomorphic species are reported as SI (East, 1940; Sobrevila and Arroyo, 1982). The occurrence of trinucleate pollen in the Boraginaceae suggests the possibility that control of SI may be sporophytic in these species. Heterostyly and homomorphic SI are well documented in the Sterculiaceae (Table 3), although phylogenetic relationships of the heterostylous and homomorphic genera are unclear.

When heteromorphic SI has been identified as the only form of SI in a plant family, it may have evolved from SC within the family, or from homomorphic SI in a more inclusive clade. Within the limitations of current phylogenetic information, most evidence suggests that heterostyly in these families evolved from self-compatible ancestors. Distyly is likely to have arisen from SC ancestors in both the Santalaceae and the Turneraceae; in the Clusiaceae it may have evolved from GSI, which is found in the related Theaceae and possibly the Lecythidaceae. For the Erythroxylaceae, Linaceae, and Oxalidaceae, the families that may be most closely related (possibly the Violaceae, Malpighiaceae, and Geraniaceae, respectively; Rodman, 1991; Chase et al., 1993) are basically SC, although there have been reports of SI in *Pelargonium* (Geraniaceae; Darwin, 1878; Sears, 1937).

The families most closely related to the Lythraceae (Punicaceae, Sonneratiaceae) show uniform SC (Graham et al., 1993), and all homomorphic genera of Lythraceae are also SC, indicating that heterostyly has been derived from SC in the Lythraceae. Based on the morphological phylogeny of Graham et al. (1993), it appears that heterostyly evolved 3–5 times within Lythraceae. Heterostyly may have been preceded by GSI in the Oleaceae, which is related to families where SI is widespread; e.g., in Bignoniaceae, where control is likely to be gametophytic on the basis of binucleate pollen and the known occurrence of GSI in the related Scrophulariaceae.

Plumbaginaceae and Polygonaceae also may have evolved heteromorphic SI from SC ancestors, on the basis of the occurrence of SC among potentially related families in the Caryophyllidae (Rodman et al., 1984; Chase et al., 1993). Plumbaginaceae is one of the few families in which the build-up of the heterostylous syndrome from a homomorphic, SC ancestor has been suggested on the basis of a comparative, though not phylogenetic, approach (Baker, 1966). Tristyly and SC are probably basal conditions in the Pontederiaceae, based on morphological and molecular evidence (Eckenwalder and Barrett, 1986; Sean Graham, pers. comm.). Among families closely related to Pontederiaceae (Chase et al., 1993), GSI is present in the Commelinaceae, but evidence for the Commelinaceae as the sister-group to the Pontederiaceae is weak (Sean Graham, pers. comm.), suggesting that GSI in Commelinaceae and heterostylous SSI in Pontederiaceae are unlikely to be evolutionarily linked.

We conclude from these considerations that heterostyly has in most cases evolved from SC precursors, possibly from ancestors with GSI in a few instances, and in all probability not from ancestors with SSI. We therefore concur with Charlesworth (1985) that it is unlikely that heteromorphic SI evolved though loss of alleles from sporophytic SI systems, as suggested by Muenchow (1982).

CONCLUSIONS

Our preliminary phylogenetic analysis favors the hypothesis that the first angiosperms were self-compatible, and implies that the evolution of SI was not a major causal factor in the radiation of flowering plants. Attempts to homologize all self-incompatibility systems in angiosperms seem unwarranted, both on the basis of the phylogenetic considerations discussed here and the evident diversity of self-incompatibility systems. There is little evidence to suggest that sporophytically controlled, homomorphic self-incompatibility evolved in species possessing gametophytic self-incompatibility. In fact, on the basis of phylogenetic reasoning, it seems more likely that both kinds of incompatibility evolved from SC ancestors. Similarly, heteromorphic self-incompatibility evolved independently on many occasions, probably from self-compatible ancestors.

Further information on breeding systems is needed to resolve the many remaining ambiguities. Despite the seven or more decades that have passed since the discovery of the genetic basis of self-incompatibility, genetic data are available for fewer than two dozen species. At an even more basic level, no information on the presence or absence of SI is available for many families. A forum for reporting observations of breeding systems, whether results are negative or positive, would be most useful (e.g., Arroyo and Weller, 1993).

Studies of the molecular basis of self-incompatibility have appeared only recently, and are still limited to only a few families (Nasrallah and Nasrallah, 1986; Haring et al., 1990; Ioerger et al., 1990; Franklin-Tong and Franklin, 1993; Newbigin et al., 1993). The molecular evidence is consistent with the view that sporophytic and gametophytic self-incompatibility evolved independently, since amino acid sequences of SI alleles from the three families for which data are available show little similarity. It may well be that each genetic type of SI includes families that employ different molecular mechanisms. This would be consistent with the results of our phylogenetic analysis. Despite recent progress in understanding the phylogeny of flowering plants, the relationships of major taxonomic groups are still poorly resolved, and few families have been studied in any detail. Better information at both levels will be essential to address evolutionary questions such as the origin of self-incompatibility. In the meantime, it is nevertheless helpful to formulate such questions in phylogenetic terms. This exercise can identify phylogenetically critical taxa whose breeding systems are as yet unknown (e.g., Barnadesinae), as well as taxa with diverse breeding systems, where detailed phylogenetic studies would be especially valuable in elucidating evolutionary pathways (e.g., Boraginaceae, Rubiaceae, Sterculiaceae). Finally, as illustrated by our analysis of the basal condition of angiosperms, it may be possible to reach some conclusions even in the face of significant ambiguity regarding phylogenetic relationships and character coding.

LITERATURE CITED

Arasu, N. T. 1970. Self-incompatibility in *Ribes*. Euphytica 19: 373–378.

Arroyo, M. T. Kalin and F. Squeo. 1990. Relationship between plant breeding systems and pollination. Pp. 205–227 *in* S. Kawano (ed.), Biological Approaches and Evolutionary Trends in Plants. Academic Press, New York.

_____ and S. G. Weller. 1993. Plant breeding system records. Plant Species Biology 8: 225.

Armbruster, W. S. 1992. Phylogeny and the evolution of plant-animal interactions. BioScience 42: 12–20.

Baker, H. G. 1965. Characteristics and modes of origin of weeds. Pp. 147–172 *in* H. G. Baker and G. L. Stebbins (eds.), The Genetics of Colonizing Species. Academic Press, New York.

_____. 1966. The evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. Evolution 20: 349–368.

_____. 1984. Some functions of dioecy in seed plants. Amer. Naturalist 124: 149–158.

Barrett, S. C. H. 1992. Heterostylous genetic polymorphisms: Model systems for evolutionary analysis. Pp. 1–29 *in* S. C. H. Barrett (ed.), Monographs on Theoretical and Applied Genetics 15. Evolution and Function of Heterostyly. Springer-Verlag, Berlin.

_____ and M. B. Cruzan. 1994. Incompatibility in heterostylous plants. *In* E. G. Williams, R. B. Knox, and A. E. Clarke (eds.), Genetic Control of Self-incompatibility and Reproductive Development in Flowering Plants. Kluwer Academic, The Netherlands. (In press).

Bateman, A. J. 1952. Self-incompatibility systems in angiosperms. Heredity 6: 285–310.

_____. 1955. Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity 9: 53–68.

Bawa, K. S. 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28: 85–92.

_____ and J. H. Beach. 1983. Self-incompatibility systems in the Rubiaceae of a tropical lowland wet forest. Amer. J. Bot. 70: 1281–1288.

_____, D. R. Perry, and J. H. Beach. 1985. Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. Amer. J. Bot. 72: 331–345.

_____ and C. J. Webb. 1983. Floral variation and sexual differentiation in *Muntingia calabura* (Elaeocarpaceae), a species with hermaphroditic flowers. Evolution 37: 1271–1282.

Bernhardt, P. and L. B. Thien. 1987. Self-isolation and insect pollination in the primitive angiosperms: new evaluations of older hypotheses. Pl. Syst. Evol. 156: 159–176.

Bond, W. J. 1989. The tortoise and the hare: ecology of angiosperm dominance and gymnosperm persistence. Biol. J. Linn. Soc. 36: 227–249.

Bremer, B. and R. K. Jansen. 1991. Comparative restriction site mapping of chloroplast DNA implies new phylogenetic relationships within Rubiaceae. Amer. J. Bot. 78: 198–213.

_____, _____, P. O. Karis, M. Källersjö, S. C. Keeley, K.-J. Kim, H. J. Michaels, J. D. Palmer, and R. S. Wallace. 1992. A review of the phylogeny and classification of the Asteraceae. Nord. J. Bot. 12: 141–148.

_____ and L. Struwe. 1992. Phylogeny of the Rubiaceae and the Loganiaceae: Congruence or conflict between morphological and molecular data. Amer. J. Bot. 79: 1171–1184.

Brewbaker, J. L. 1957. Pollen cytology and self-incompatibility systems in plants. J. Hered. 48: 271–277.

_____. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. Amer. J. Bot. 54: 1069–1083.

Brooks, D. R. and D. A. McLennan. 1991. Phylogeny, Ecology, and Behavior. Univ. Chicago Press, Chicago.

Bullock, S. H. 1985. Breeding systems in the flora of a tropical deciduous forest in Mexico. Biotropica 17: 287–301.

Carlquist, S. 1974. Island Biology. Columbia Univ. Press, New York.

Charlesworth, D. 1979. The evolution and breakdown of tristyly. Evolution 33: 486–498.

_____. 1982. On the nature of the self-incompatibility locus in homomorphic and heteromorphic systems. Amer. Naturalist 119: 732–735.

_____. 1985. Distribution of dioecy and self-incompatibility in angiosperms. Pp. 237–268 *in* J. J. Greenwood and M. Slatkin (eds.), Evolution—Essays in Honour of John Maynard Smith. Cambridge Univ. Press, Cambridge, U.K.

Chase, M. W. et al. (37 other authors). 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528–580.

Crane, P. R. 1989. Early fossil history and evolution of Betulaceae. Pp. 87–116 *in* P. R. Crane and S. Blackmore (eds.), Evolution, Systematics, and Fossil History of the Hamamelidae. Volume 2: 'Higher' Hamamelidae. Clarendon Press, Oxford, U.K.

Cronquist, A. 1988. The Evolution and Classification of Flowering Plants. The New York Botanical Garden, New York.

Crowe, L. K. 1954. Incompatibility in *Cosmos bipinnatus*. Heredity 8: 1–11.

_____. 1964. The evolution of outbreeding in plants I. The angiosperms. Heredity 19: 435–457.

_____. 1971. The polygenic control of outbreeding in *Borago officinalis*. Heredity 27: 111–118.

Dahlgren, R. and K. Bremer. 1985. Major clades of the angiosperms. Cladistics 1: 349–368.

Darwin, C. 1877. The Different Forms of Flowers on Plants of the Same Species. John Murray, London.

_____. 1878. The Effects of Cross and Self Fertilisation in the Vegetable Kingdom. John Murray, London.

de Nettancourt, D. 1977. Monographs on Theoretical and Applied Genetics 3. Incompatibility in Angiosperms. Springer-Verlag, Berlin.

Donoghue, M. J. 1989. Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. Evolution 43: 1137–1156.

_____ and J. A. Doyle. 1989. Phylogenetic analysis of angiosperms and the relationships of "Hamamelidae." Pp. 17–45 *in* P. R. Crane and S. Blackmore (eds.), Evolution, Systematics and Fossil History of the Hamamelidae. Volume 2: 'Higher' Hamamelidae. Clarendon, Oxford, U.K.

Doyle, J. A. and M. J. Donoghue. 1986. Seed plant phylogeny and the origin of angiosperms: an experimental cladistic approach. Bot. Rev. 52: 321–431.

_____ and _____. 1993. Phylogenies and angiosperm diversification. Paleobiology 19: 141–167.

_____ and C. L. Hotton. 1991. Diversification of early angiosperm pollen in a cladistic context. Pp. 169–195 *in* S. Blackmore and S. H. Barnes (eds.), Pollen and Spores: Patterns of Diversification. Clarendon, Oxford, U.K.

Dulberger, R. 1970. Floral dimorphism in *Anchusa hybrida* Ten. Isr. J. Bot. 19: 37–41.

East, E. M. 1940. The distribution of self-sterility in the flowering plants. Proc. Amer. Philos. Soc. 82: 449–518.

_____ and A. J. Mangelsdorf. 1925. A new interpretation of the hereditary behaviour of self-sterile plants. Genetics 2: 505–609.

Eckenwalder, J. E. and S. C. H. Barrett. 1986. Phylogenetic systematics of Pontederiaceae. Syst. Bot. 11: 373–391.

Endress, P. K. 1984. The flowering process in the Eupomatiaceae (Magnoliales). Bot. Jahrb. Syst. 104: 297–319.

_____. 1987. The Chloranthaceae: reproductive structures and phylogenetic position. Bot. Jahrb. Syst. 109: 153–226.

Ervik, F., S. S. Renner, and K. A. Johanson. Breeding system pollination of *Nuphar luteum* (L.) Smith (Nymphaeaceae) in Norway. Flora (in press).

Franklin, E. C. 1970. Survey of mutant forms and inbreeding depression of the family Pinaceae. U. S. D. A. Forest Service Research Paper SE 61.

Franklin-Tong, V. E. and F. C. H. Franklin. 1993. Gametophytic self-incompatibility: contrasting mechanisms for *Nicotiana* and in *Papaver*. Trends Cell Biol. 3: 340–345.

Free, J. B. 1970. Insect Pollination of Crops. Academic Press, London.

Fryxell, P. A. 1957. Mode of reproduction of higher plants. Bot. Rev. (Lancaster) 23: 135–233.

Ganders, F. R. 1976. Self-incompatibility in the Cactaceae. Cact. Succ. J. Gr. Brit. 38: 39–40.

_____. 1979. The biology of heterostyly. New Zealand J. Bot. 17: 607–635.

Gerstel, D. U. 1950. Self-incompatibility studies in Guayule II. Inheritance. Genetics 35: 482–506.

Gibbs, P. E. 1986. Do homomorphic and heteromorphic self-incompatibility systems have the same sporophytic mechanism? Pl. Syst. Evol. 154: 285–323.

_____. 1988. Self-incompatibility mechanisms in flowering plants: some complications and clarifications. Lagascalia 15: 17–28.

Godley, E. J. and D. H. Smith. 1981. Breeding systems in New Zealand plants 5. *Pseudowintera colorata* (Winteraceae). New Zealand J. Bot. 19: 151–156.

Gornall, R. J. and B. A. Bohm. 1984. Breeding systems and relationships among species of *Boykinia* and related genera (Saxifragaceae). Canad. J. Bot. 62: 33–37.

Gottsberger, G. 1977. A cantarofilia em Talauma ovata (Magnoliaceae). XXVIII Congr. Nac. Bot., Belo Horizonte, Minas Gerais, 23–30 Jan. 1977. Abstr. Sessao Tecnica II, 14.

_____, I. Silberbauer-Gottsberger, and F. Ehrendorfer. 1980. Reproductive biology in the primitive relic angiosperm *Drimys brasiliensis* (Winteraceae). Pl. Syst. Evol. 135: 11–39.

Graham, S. A., J. V. Crisci, and P. C. Hoch. 1993. Cladistic analysis of the Lythraceae *sensu lato* based on morphological characters. Bot. J. Linn. Soc. 113: 1–33.

Gupta, S., R. Seed, K. Prabha, and S. C. Gupta. 1981. Overcoming self-incompatibility in *Argyreia campanulata* by Rifampicin and cycloheximide. Incompat. Newslett. 13:61–68.

Hagman, M. 1975. Incompatibility in forest trees. Proc. Roy. Soc. London Ser. B, Biol. Sci. 188: 313–326.

Hamby, R. K. and E. A. Zimmer. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. Pp. 50–91 *in* P. S. Soltis, D. E. Soltis, and J. J. Doyle (eds.), Molecular Systematics of Plants. Chapman and Hall, New York.

Haring, V., J. E. Gray, B. A. McClure, M. A. Anderson, and A. E. Clarke. 1990. Self-incompatibility: A self-recognition system in plants. Science 250: 937–941.

Heiser, C. B., Jr. 1962. Some observations on pollination and compatibility in *Magnolia*. Proc. Indiana Acad. Sci. 72: 259–266.

Hughes, M. B. and E. B. Babcock. 1950. Self-incompatibility in *Crepis foetida* L. subsp. *rhoedaifolia*. Genetics 35: 570–588.

Hufford, L. D. 1992. Rosidae and their relationships to other nonmagnoliid dicotyledons: A phylogenetic analysis using morphological and chemical data. Ann. Missouri Bot. Gard. 79: 218–248.

_____ and P. R. Crane. 1989. A preliminary phylogenetic analysis of the 'lower' Hamamelidae. Pp. 175–192 *in* P. R. Crane and S. Blackmore (eds.), Evolution, Systematics and Fossil History of the Hamamelidae. Volume 1: Introduction and 'Lower' Hamamelidae. Clarendon Press, Oxford.

Humphreys, M. O. and J. S. Gale. 1974. Variation in wild populations of *Papaver dubium*. VIII. The mating system. Heredity 33: 33–41.

Ioerger, T. R., A. E. Clark, and T-H. Kao. 1990. Polymorphism at the self-incompatibility locus in Solanaceae predates species. Proc. Nat. Acad. Sci. U.S.A. 87: 9732–9735.

Jacob, V. J. 1980. Pollination, fruit setting and incompatibility in *Cola nitida*. Incompat. Newslett. 12: 50–56.

Joseph, C. A. and M. K. George. 1983. Fruit and seed set in sweet potato (*Ipomoea batatas* (L.) Lam.). Incompat. Newslett. 15: 4–7.

Judd, W., R. Sanders, and M. J. Donoghue. 1994. Angiosperm family pairs: preliminary phylogenetic analyses. Harvard Pap. Bot. 5: 1–51.

Knight, R. and H. H. Rogers. 1955. Incompatibility in *Theobroma cacao*. Heredity 9: 69–77.

Kowyama, Y., N. Shimano, and T. Kawasi. 1980. Genetic analysis of incompatibility in the diploid species of *Ipomoea* closely related to the sweet potato. Theor. Appl. Genet. 58: 149–155.

Krebs, S. L. and J. F. Hancock. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry *Vaccinium corymbosum* L. Theor. Appl. Genet. 79: 825–832.

Kubitzki, K. and H. Kurz. 1984. Synchronized dichogamy and dioecy in neotropical Lauraceae. Pl. Syst. Evol. 147: 253–266.

Lawrence, M. J. 1975. The genetics of self-incompatibility in *Papaver rhoeas*. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 188: 275–285.

Lewis, D. 1977. Sporophytic incompatibility with 2 and 3 genes. Proc. Roy. Soc. London Ser. B, Biol. Sci. 196: 161–170.

Lloyd, D. G. and C. J. Webb. 1992. The evolution of heterostyly. Pp. 151–178 *in* S. C. H. Barrett (ed.), Monographs on Theoretical and Applied Genetics 15. Evolution and Function of Heterostyly. Springer-Verlag, Berlin.

_____, _____, and R. Dulberger. 1990. Heterostyly in species of *Narcissus* (Amaryllidaceae) and *Hugonia* (Linaceae) and other disputed cases. Pl. Syst. Evol. 172: 215–227.

_____ and M. S. Wells. 1992. Reproductive biology of a primitive angiosperm, *Pseudowintera colorata* (Winteraceae), and the evolution of pollination systems in the Anthophyta. Pl. Syst. Evol. 181: 77–95.

Loconte, H. and D. W. Stevenson. 1991. Cladistics of the Magnoliidae. Cladistics 7: 267–296.

Lorence, D. H. 1985. A monograph of the Monimiaceae (Laurales) in the Malagasy region (southwest Indian Ocean). Ann. Missouri Bot. Gard. 72: 1–165.

Lundqvist, A. 1975. Complex self-incompatibility in angiosperms. Proc. Roy. Soc. London Ser. B, Biol. Sci. 188: 235–245.

_____. 1990. One-locus sporophytic S-gene system with traces of gametophytic pollen control in *Cerastium arvense* subsp. *strictum* (Caryophyllaceae). Hereditas 113: 203–215.

Maddison, W. P., M. J. Donoghue, and D. R. Maddison. 1984. Outgroup analysis and parsimony. Syst. Zool. 33: 83–103.

_____ and D. R. Maddison. 1992. MacClade: Interactive Analysis of Phylogeny and Character Evolution, Vers. 3.0. Sinauer Assoc., Sunderland, Mass.

McDade, L. A. 1992. Pollinator relationships, biogeography, and phylogenetics. BioScience 42: 21–26.

McDaniel, J. C. 1963. Securing seed production in *Magnolia acuminata* and *M. cordata*. Proc. Int. Pl. Propag. Soc. 13: 120–123.

Moore, D. M. 1968. The vascular flora of the Falkland Islands. Sci. Rep. Brit. Antarc. Surv. 60: 1–202.

Muenchow, G. 1981. An S-locus model for the distyly supergene. Amer. Naturalist 118: 756–760.

_____. 1982. A loss-of-alleles model for the evolution of distyly. Heredity 49: 81–93.

Nasrallah, M. E. and J. B. Nasrallah. 1986. Molecular biology of self-incompatibility in plants. Trends Gen. 2: 239–244.

Newbigin, E., M. A. Anderson, and A. E. Clarke. 1993. Gametophytic self-incompatibility systems. Plant Cell 5: 1315–1324.

Nixon, K. C. and J. I. Davis. 1991. Polymorphic taxa, missing values, and cladistic analysis. Cladistics 7: 233–241.

Norton, R. A., J. B. Kethley, D. E. Johnson, and B. M. O'Connor. 1993. Phylogenetic perspectives on genetic systems and reproductive modes in mites. Pp. 8–99 *in* D. Wrensch and M. Ebbert (eds.), Evolution and Diversity of Sex Ratio in Insects and Mites. Chapman and Hall, London.

Olmstead, R. G. 1989. The origin and function of self-incompatibility in flowering plants. Sexual. Pl. Reprod. 2: 127–136.

_____, H. J. Michaels, K. M. Scott, and J. D. Palmer. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbc*L. Ann. Missouri Bot. Gard. 79: 249–265.

_____, B. Bremer, K. M. Scott, and J. D. Palmer. 1993. A parsimony analysis of the Asteridae *sensu lato* based on *rbc*L sequences. Ann. Missouri Bot. Gard. 80: 700–722.

Ornduff, R. 1974. Heterostyly in South African flowering plants: a conspectus. J. S. African Bot. 40: 169–187.

_____. 1988. Distyly and monomorphism in *Villarsia* (Menyanthaceae): some evolutionary considerations. Ann. Missouri Bot. Gard. 75: 761–767.

Østerbye, U. 1977. Self-incompatibility in *Ranunculus acris* L. II. Four S-loci in a German population. Hereditas 87: 173–178.

Parks, C. R., N. G. Miller, J. F. Wendel, and K. M. McDougal. 1983. Genetic divergence within the genus *Liriodendron* (Magnoliaceae) Ann. Missouri Bot. Gard. 70: 658–666.

Pojar, J. 1974. Reproductive dynamics of four plant communities of southwestern British Columbia. Canad. J. Bot. 52: 1819–1834.

Prakash, N. and J. H. Alexander. 1984. Self-incompatibility in *Austrobaileya scandens*. Pp. 214–216 *in* E. G. Williams and R. B. Knox (eds.), Pollination '84. School of Botany, University of Melbourne, Australia.

Prance, G. G. and A. B. Anderson. 1976. Studies of the floral biology of neotropical Nymphaeaceae 3. Acta Amazon. 5: 109–170.

Rodman, J. E. 1991. A taxonomic analysis of glucosinolate-producing plants, Part 2: Cladistics. Syst. Bot. 16: 619–629.

_____, M. K. Oliver, R. R. Nakamura, J. U. McClammer, and A. H. Bledsoe. 1984. A taxonomic analysis and revised classification of Centrospermae. Syst. Bot. 9: 297–323.

Rust, R. W. and R. R. Roth. 1981. Seed production and seedling establishment in the mayapple, *Podophyllum peltatum* L. Amer. Midl. Naturalist 105: 51–60.

Sarvas, R. 1962. Investigations of the flowering and seed crop of *Pinus sylvestris*. Commun. Inst. Forest. Fenniae 53: 1–198.

Schneider, E. L. and J. M. Jeter. 1982. Morphological studies of the Nymphaeaceae. XII. The floral biology of *Cabomba caroliniana*. Amer. J. Bot. 69: 1410–1419.

_____ and L. A. Moore. 1977. Morphological studies of the Nymphaeaceae. VII. The floral biology of *Nuphar lutea* subsp. *macrophylla*. Brittonia 29: 88–99.

Schou, O. and M. Philipp. 1983. An unusual heteromorphic incompatibility system. II. Pollen tube growth and seed sets following compatible and incompatible crossings within *Anchusa officinalis* L. (Boraginaceae). Pp. 219–227 *in* D. L. Mulcahy and E. Ottaviano (eds.), Pollen: Biology and Implications for Plant Breeding. Elsevier, New York.

Scribailo, R. W. and S. C. H. Barrett. 1991. Pollen-pistil interactions in tristylous *Pontederia sagittata* (Pontederiaceae). II. Patterns of pollen tube growth. Amer. J. Bot. 78: 1662–1682.

Sears, E. R. 1937. Cytological phenomena associated with self-sterility in the flowering plants. Genetics 22: 130–181.

Semple, K. S. 1974. Pollination in Piperaceae. Ann. Missouri Bot. Gard. 61: 868–871.

Snigirevskaya, N. S. 1964. Contributions to the morphology and taxonomy of the genus *Nelumbo* Adan. Tr. Bot. Inst. Komarov. Akad. Nauk SSR Ser. I. 13: 104–172.

Sobrevila, C. and M. T. Kalin Arroyo. 1982. Breeding systems in a montane tropical cloud forest in Venezuela. Pl. Syst. Evol. 140: 19–37.

Sohmer, S. H. 1977. Aspects of the biology of *Nelumbo pentapetala* (Walter) Fernald, the American lotus, on the upper Mississippi. Wis. Acad. Sci., Arts Letters. 65: 258–273.

Soltis, D. E., D. R. Morgan, A. Grable, P. S. Soltis, and R. Kuzoff. 1993. Molecular systematics of Saxifragaceae sensu stricto. Amer. J. Bot. 80: 1056–1081.

Sorensen, F. C. 1982. The roles of polyembryony and embryo viability in the genetic systems of conifers. Evolution 36: 725–733.

Stebbins, G. L. 1957. Self fertilization and population variability in the higher plants. Amer. Naturalist 91: 337–354.

_____. 1974. Flowering Plants Evolution Above the Species Level. Harvard Univ. Press, Cambridge, Massachusetts.

Stevens, V. A. M. and B. G. Murray. 1982. Studies on heteromorphic self-incompatibility systems: physiological aspects of the incompatibility system of *Primula obconica*. Theor. Appl. Genet. 61: 245–256.

Stout, A. B. 1923. Alternation of sexes and intermittent production of fruit in the spider flower (*Cleome spinosa*). Amer. J. Bot. 10: 57–67.

Suzuki, K. 1983. Breeding system and crossability in Japanese *Epimedium* (Berberidaceae). Bot. Mag. (Tokyo) 96: 343–350.

Takhtajan, A. 1991. Evolutionary Trends in Flowering Plants. Columbia Univ. Press, New York.

Tanaka, H. and T. Yahara. 1989. Self-pollination of *Asarum caulescens* Maxim. (Aristolochiaceae) in Japan. Pl. Spec. Biol. 2: 133–136.

Taroda, T. and P. E. Gibbs. 1982. Floral biology and breeding system of *Sterculia chicha*. St. Hil. New Phytol. 90: 735–743.

Taylor, D. W. and L. J. Hickey. 1992. Phylogenetic evidence for the herbaceous origin of angiosperms. Pl. Syst. Evol. 180: 137–156.

Thien, L. B. 1980. Patterns of pollination in the primitive angiosperms. Biotropica 12: 1–13.

_____, D. A. White, and L. Y. Yatsu. 1983. The reproductive biology of a relict—*Illicium floridanum* Ellis. Amer. J. Bot. 70: 719–727.

Thompson, M. M. 1979. Genetics of incompatibility in *Corylus avellana* L. Theor. Appl. Genet. 54: 113–116.

Tomlinson, P. B. 1974. Breeding mechanisms in trees native to tropical Florida—a morphological assessment. J. Arnold Arbor. 55: 269–290.

Vithanage, H. I. M. 1984. Pollen-stigma interactions: development and cytochemistry of stigma papillae and their secretions in *Annona squannosa* L. Ann. Bot. (London) 54: 153–167.

Wheelwright, N. T. 1985. Competition for dispersers and the timing of flowering and fruiting in a guild of tropical trees. Oikos 44: 465–477.

Whitehouse, H. L. K. 1950. Multiple-allelomorph incompatibility of pollen and style in the evolution of the angiosperms. Ann. Bot. 14: 199–216.

Wildman, H. E. 1950. Pollination of *Asarum canadense* L. Science 111: 551.

Zadoo, S. N. and T. N. Khoshoo. 1975. Self-incompatibility in *Bougainvillea*. Incompat. Newslett. 5: 73–75.

Zapata, T. R. and M. T. Kalin Arroyo. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10: 221–230.

Zavada, M. S. 1984. The relation between pollen exine sculpturing and self-incompatibility mechanisms. Pl. Syst. Evol. 147: 63–78.

abscisic acid, 254
Acanthaceae, 314, 374
Acorus, 27, 29–30, 36–37
adder's-tongue fern (*Ophioglossum petiolatum*), 135
Adh1. *See* alcohol dehydrogenase.
Adiantum, 119–121
adnation, 160
Aglaomorpha, 126, 132, 135, 137
Aglaophyton, 79, 81
Agrostis, 178–179
alcohol dehydrogenase (*Adh1*), 51–52
Aletris, 32
Alfaroa, 195
Alismatanae, 30, 36–37
allopolyploidy, 305, 309–310, 321–322, 334, 342, 344
allorrhizic, 112; allorhizy, 114
allozymes, xiv, 7, 59, 215–217, 219
Alnus, 370
Amaryllidaceae, 29, 373–374
Amblystegiaceae, 103
Amborella, 362, 364
Ambrosia, 205
amino acid sequences, xiv, xvii, 50, 53, 376
amphibians, 309
Anabaena, 135
anagenesis, 263, 268–270, 286
Ananas, 137, 357
Anas, 269
Andreaeidae, 89, 92–93
Andreaea, 93, 98
Anemia, 121, 137–139
Aneura, 90
Annonaceae, 9, 361–365
Anthocercis, 158
Anthocleista, 148, 150
Anthoxanthum, 209
Aphelandra, 314–315, 319, 333, 346
Apocynaceae, 159, 162
Apometzgeria, 90
Aptian, 190
Araceae, 27, 29, 37, 137–138
Arachis, 267
Araliaceae, 162
Aranae, 29–30, 36–37
Araucaria, 4, 147–148, 152–153, 155
Archaeanthus, 191–192
architectural models, viii, 147–148, 150–156, 157–171
Arecaceae, 27, 32, 34, 153
Arecanae, 30, 35, 37
Argyreia, 372
Aristolochiaceae, 362, 364
Asarum, 364
Asclepias, 226–228, 235, 250
Aspargales, 29–30, 32, 37
Asplenium, 121, 124, 126, 131
Asteraceae, v, xiv, 15–22 (*Microseris*), 28, 357, 368, 370–371, 373
Asteridae, 28, 362, 370
Aulacomnium, 103
Austrobaileya, 362–363, 365
Austrobaileyaceae, 363
Azolla, 130, 135–136, 141

backcross, 61, 309–310, 325
bacteria. See *E. coli*, *Rhizobium*.
Balsaminaceae, 201
Bambusa, 29, 37
Baptisia, xiii
Barnedesinae, 370
Barro Colorado Island, 223–225
Bartramiaceae, 103
basal taxa, 289–291
Bauhinia, 374
Bauplan, 111–116, 130, 139, 141. *See also* ground plan.
bees, 1, 194, 265; bumblebees, 225
Belliolum, 363
Berberidaceae, 360, 362
Betula, 9, 370
Betulaceae, 9, 357, 368, 370–373
Bignoniaceae, 375
biogeography, xvi–xviii, 1, 4, 6–7, 272, 321; historical biogeography, xvi, 321
biosynthetic pathway, 314
biosystematics, definition of, xii
biparental inbreeding, 234
bird's nest fern (*Asplenium nidus*), 126, 131
birds, 187, 194, 265, 309
Black Beauty Bush cultivar (zucchini), 233–239
Blattknospe, 123
Blechnaceae, 112, 115
Bolbitis, 121
bootstrap, 4, 20, 30, 32–33, 37
Boraginaceae, 162, 370, 374–376
Borago, 370
Botrychium, 120, 137, 139, 142
bottlenecks, 266, 293–294
Bougainvillea, 370–371
Brachythecium, 97–98, 100

branching: complanate branching, 100, 103; dichasial branching, 160, 162, 167–169; monochasial branching, 160–162, 167–169; monopodial branching, 71, 88–90, 94, 124, 147, 152, 158, 161, 170–171; patterns, viii, 71–84, 87–105, 157–171, 173–185, 280, 285, 307; radial branching, 103; sympodial branching, 71, 91, 93–94, 101, 124, 147, 152, 156, 158–160, 171
Brassica, 222, 248
Brassicaceae, 357–358, 368, 370–373
breeding systems, xiii, 217–220, 226, 229, 263–270, 355–376
Bromelianae, 29–30, 35, 38
Brugmansia, 160
Bryaceae, 94–95, 103
Bryidae, 89, 92–94
bryophytes, 73, 75, 78, 87–105
Bryophyte Model. *See* Models of Growth.
Bryum, 94, 103
Buchloe, 268–269
buffalo grass (*Buchloe dactyloides*), 268–269
Butomaceae, 189

Cabomba, 362, 364
Cabombaceae, 362, 364
Cactaceae, 139, 368, 379
Calliergonella, 103
Callistemon, 137
Callitriche, 266
Calobryales, 89, 91
Caloda, 191, 193–194
Calycanthaceae, 362, 364
Campanula, 252
Campsis, 235–236, 251
Camptosorus, 120–123
Canellaceae, 362, 364
Capparaceae, 370–371
Caprifoliaceae, 368
Capsicum, 160
Carboniferous, 73, 82–84, 87
carnivorous plant, 27
Caryophyllidae, 362, 370, 375
Caryophyllanae, 28
Cassia, 246, 249, 256
Catasetum, 269
Catostomidae, 309. *See also* fish.
Cenozoic, 188–189
Cerastium, 370–371
Ceratophyllum, 27, 32, 190
Ceratopteris, 130
Cercidiphyllum, 6
Cestroideae, 158, 160, 162, 165–166
Cestrum, 158
Cheilothela, 104
Chenopodiaceae, 357–358
Chisocheton, 126
Chloranthaceae, 362, 364
chloroplast DNA (cpDNA), xiv, xvi–xvii, 1, 3, 7–8, 15, 17, 22, 229, 267, 320–321, 334–336, 340–345, 353, 373
chromosome inversion, 284
Cinclidotus, 96, 104
Circaea, xvii–xviii
cladistic intermediacy, 317
cladistics, xi, xv, 17–21, 187, 264, 300, 305–326, 333, 337–338, 351. *See also* phylogenetic systematics.
Clarkia, xiv, xvi; section *Sympherica*, xvi
Clarkia, Idaho, 1–4, 6–8, 10
Cleome, 370
Climacium, 98, 101, 103–104
clonal herbs, 173–174. See *Trifolium repens* and *Glechoma hederacea*.
closed carpels, 187, 189–191, 195
cloudwater, 100–101
Clusiaceae, 374–375
coadapted gene complex, 265
coalescence, 285, 292, 295–301; genetic coalescence, 292, 300
coalescent theory, 280, 282, 289, 295–301
Cocculus, 6, 9
coevolution, 187–188, 194, 233
Cola, 373
Commelinaceae, 29–30, 357, 375
competition, 68, 98, 100, 148, 179–180, 183–185, 191, 237–239, 267
concaulescence. *See* adnation
concerted evolution, 47, 312
Conocephalum, 90, 98
conservation biology, 230, 266
Convolvulaceae, 357, 368, 371–373
Cooksonia, 74, 79–80
Cooksonioid Model. *See* Models of Growth.
Coptis, 189
corals, 148, 153–154
Corylus, 357, 370–371
cottonwood, 321
Cotyledonoid Model. *See* Models of Growth.
coyotes, 313, 321
cpDNA. *See* chloroplast DNA.
Cratoneuron, 103
Cretaceous, 1, 6, 10, 82, 187–191, 194–195
Cruciferae, 265, 377
Cryphaea, 104
Cryptantha, 255
CTAB extraction procedure, 3, 8
Cucurbita, 228; *C. pepo*, 233–239
Cunninghamia, 7
Cuphea, 374

Cupressaceae, 3–5, 7
cycads, 188, 364
Cycadeoidea, 188
Cyclanthanae, 29–30, 32, 37
Cycnoches, 269
Cyperales, 29–30, 38
Cyphomandra, 159
cytoplasmic genetic effects, 206
cytoplasmic inheritance, 313

Dactylis, 179–180
database, xii, 358
Datura, 159, 161
Dawsonia, 96, 101, 104
decay index, 4, 32
Degeneria, 189, 362, 364
Degeneriaceae, 364
Dendroceros, 88
Dendroligotrichum, 98, 103
Dennstaedtiaceae, 124, 128
developmental constraints, 87, 105, 111, 140–141, 199–201, 209
developmental homeostasis, 209
Devonian, 73–74, 78, 80–83, 87, 188–189
dichogamy, 313
Dicranopteris, 124, 128
dioecious, 235, 269, 364; dioecism, 268; dioecy, 268, 364, 370, 373
Dioscoreales, 30
Diospyros, 9, 147, 152
diploidization, 325
Dipsacales, 27
distyly, 374–375
DNA: fingerprinting, 15, 19, 21. *See* chloroplast DNA (cpDNA), fossil DNA, nuclear DNA markers–restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), nuclear ribosomal DNA (rDNA), selfish DNA.
dormancy, 206
Draba, 265
Drimys, 189, 379
Drosophila, 46, 49
dry stigmas, 356
Drynaria, 126, 130, 133, 135, 141

E. coli, 8, 48
effective population size, 265, 273, 281–283, 296
embryophytes, 71–73, 75, 78–80
endosperm nuclear parental effects, 206
Eocene, 187–188, 194–195
Eospermatopteris, 83
Epilobieae, xvii
Epilobium, xvii, 173, 256
Equisetum, 81
Ericaceae, 374
Erythroxylaceae, 374–375
eudicots, 360
Euphorbia, 147, 151, 158
Euphorbiaceae, 162
Eupomatiaceae, 362, 364
exclusivity, 285, 292–300
Exospermum, 363
extinction, viii, xi–xii, 1, 189, 276, 280, 300, 323–325, 368

Fabaceae, 9, 158, 267, 357, 374. *See also* Leguminosae.
Fadyenia, 121
filmy ferns, 104
fish, 309, 323–325; cypriniform, 309, 324
Fissidens, 103
Fitch-Margoliash procedure, 4
fitness, 205–210
Flagellaria, 38
flower: abortion, 248, 251–254; evolution, 189–195; position, 187, 189, 191–195; symmetry, 187, 189, 194
Fossils: viii, xv–xvi, 1–10, 27, 32, 52, 71, 75, 78–81, 83–84, 187–191, 194–195, 300, 358; fossil DNA, xv, 1–10; fossil flowers, 188, 191–194; fossil record, 7, 32, 81, 87, 104, 187–195
founder population, 293
Freesia, 374
Fremontodendron, 158–159
frequency-dependent selection, 296
frugivores, 325
fruit: abortion, 234–236, 248, 251–252, 254, 257; dispersal, 187–188, 194–195; maturation, 233–234, 239, 250–251, 257
Frullania, 98, 101
Fuchsia, xvii

Galium, 357, 371–373
Gayophytum, xvii
Geissorhiza, 374
gene flow, xiv, 16, 20–21, 195, 215–230, 263, 265, 273, 281
gene tree, 273–286, 297–298, 333–346
genealogical species. *See* species concepts, genealogical.
genetic drift, 215, 219, 221, 227, 229
genetic "sieve", 239
genetic structure, viii, 215, 219, 227, 229
genotype–environment interaction, 199–210
Gentianaceae, 373–374
Geraniaceae, 375
germination, 121, 187, 206, 208, 210, 236

Gilia, 333–338, 344, 348
Ginkgo, 364
Gladiolus, 374
Glechoma hederacea, 173–177, 181–185
Gleicheniaceae, 124, 128
Gnetales, 32, 364
Gossypium, 56, 321
grasses, 37, 41–53, 75, 175, 179, 184, 268–269, 358
Grimmia, 95, 98–100
Grimmiaceae, 95, 103
ground plan, 111–112, 115, 137, 139–140. *See also* Bauplan.
growth: acroplastic, 111; determinate, 75–76, 78, 80, 83, 120, 126, 158, 161, 170–171, 174, 248; indeterminate, 76, 78, 80–83, 123, 126, 158, 171, 233; stoloniferous, 111, 113, 120–124. *See also* Models of Growth.
Guarea, 126
Gymnomitrion, 101
Gymnostachys, 29, 37

habit: epiphytic, 111–113, 126–131, 134, 137; floating aquatic, 111–113, 130–136; vining, 111–113, 123–126
Haemodorales, 30
Hamamelidales, 360–365
Hamelieae, 372, 375
Haplomitrium, 104
Hauya, xvii
Hauyeae, xvii
Herberta, 98, 101
Hedeia, 80
Helianthus, 299, 312–313, 333–353; section *Helianthus*, 335, 336, 339, 341, 343, 349, 352–353
Hemitrapa, 190
hepatics, 78, 87
herbivores, 325–326
heritable variation, 238
hermaphroditic flowers, 269. *See also* species, hermaphroditic.
Hernandiaceae, 360, 362
heterodichogamy, 363
Heterogaura, xvi
heterospory, 81–82, 189
heterostyly, 373–375
Hibiscus, 239, 372
Himantandraceae, 362, 364
Histiopteris, 124, 128
Holcus, 178–179
homoplasy, 20, 305, 315, 317, 319, 324, 346
homorrhizic, 112, 114–115 (homorrhizy)
hopeful monster, 325
hormones, 235, 254
Horneophyton, 81
hornworts, 75, 78, 88–90
human diaspora, 325
human population, xi
hybrid, 59, 61–64 (cross between maize and teosinte), 68, 233–239 (zucchini cultivar), 299, 333–346; derived hybrids, 305, 310, 312, 319–324; hybrid, autapomorphic, 311, 314, 317; hybrid bridge hypothesis, 326; hybrid character expression, 344; hybrid species, 310, 323, 333–346; homoploid hybrid species, 333–334, 345; hybrid zones, 321, 326; primary hybrids, 305, 310, 312, 321–324
hybridization, viii, xiv, 15–17, 21, 49–50, 263, 266, 274, 278, 299, 305–326, 333–336, 342–346; definition of, 307; delimitation of, 305
Hydrocharitaceae, 189
Hylocomium, 97–98, 100–101
Hymenophyllum, 104
Hymenophyton, 90, 98, 104
Hyoscyamnus, 160
Hypolepis, 112, 116
hyponasty, 117, 119, 123

Icanthaceae, 159
Illiciaceae, 363, 365
Illicium, 362–363
Impatiens, 199, 201–203, 205
inbreeding depression, 234, 357, 363, 370
independent assortment, 278–279
interbreeding units, 289, 301
internal geitonogomy, 266
introgression, xiv, 22, 52, 313,
Ipomoea, 357, 371–372
Iridaceae, 29, 37, 373, 374
Isoetoid Model. *See* Models of Growth.
isolating barriers, 264
Isopterygium, 93
Isothecium, 104
isozymes, 266, 311–313, 325, 347

Jaltomata, 162, 265, 272
Jepsonia, 375
jewelweed (*Impatiens capensis*), 201–202
Joinvillea, 29, 38
Juglandaceae, 195
Jungermanniales, 89, 91–92, 104
Jurassic, 188

karyology, xiii, 305
Koonwarra Formation (Australia), 190–191
Kurzia, 104

Lactoridaceae, 362, 364

Lamiaceae, 174, 265
Lardizabalaceae, 360, 362
Lathyrus, 226, 228
Lauraceae, 9, 360, 362–363
Laurales, 360
Leavenworthia, 266
Lecythidaceae, 375
Leguminosae, 9, 174, 195, 234, 246. *See also* Fabaceae.
Lejeunea, 91, 101
Lemna, 29, 130, 135
Lepidodendron, 75
Lepidosigillaria, 83
Leptoglossis, 162
leptokurtic, 222, 224
Leptostemonum, 270
Lesqueria, 191–192
life-history, 139
Liliaceae, 9, 162, 357
Lilianae, 29–30, 32, 36–38
Linaceae, 374–375
Lindera, 9, 235
Linnaeus' "sexual system", 263
Liquidambar, 2, 7, 9
Liriodendron, 7, 9
Lisianthius, xiv
liverworts, 4, 75, 78–79, 88–92, 101, 104–105; thalloid liverworts, 88–90, 98
Lobelia, 254
Lobeliaceae, 368, 371
Loganiaceae, 147–148, 150, 373–374, 377
Lolium, 178–179, 184
Lopezia, xvii–xviii
Lophocolea, 100
Lotus, 234–236
Ludwigia, xvii
Lupinus, 222
Lycieae, 159
Lycianthes, 160, 168
Lycopersicum, 160
Lycopodium, 74–75, 137
Lycopsida, 135, 137
Lygodium, 111, 123–127, 129
Lythraceae, 374–375

MacClade, 358, 361, 366–367, 369
macromolecules, xiii
Magnolia, 1, 3, 4, 9, 11, 27, 363; *M. latahensis*, 1, 3–4
Magnoliidae, 360
maize, 3, 41–45, 49–53, 57–69, 148
mallard ducks, 269
Malpighiaceae, 375
mammals, 194, 309
Marchantia, 4
Marsileaceae, 130
Marsupella, 98, 101
marsupial wolf, 1
maternal phenotypic effects, 206–209, 238–239
maximum likelihood procedures/methods, 4, 27–28, 30–33, 282
Medicago, 256
Medullosa, 188
Megaceros, 88
Meliaceae, 126
Menispermaceae, 6, 9, 360, 362
Menyanthaceae, 371, 374
Mesozoic, 188–189, 268
Metasequoia, 3, 7, 9
metaspecies, 297
Metzgeriales, 89–90, 104
mice, 321
Microgonium, 120
Microgramma, 117–118, 120
micropolyphyly, 321
Microseris, v, 15–22; *M. pygmaea*, 15, 17–22; *M. scapigera*, 15–18, 21–22
migration, 280–281
milkweed, 226–227
Miocene, 1–10
mixed-mating, 218–220
Mniaceae, 96, 101–103
Models of Growth, 71–84, 147–156; Bryophyte Model, 71–73, 76, 78–81; Cooksonioid Model, 71–73, 76, 80–83; Cotyledonoid Model, 71–73, 77, 82–84; Isoetoid Model, 71–73, 77, 82–84; Psilotioid Model, 71–73, 76, 77, 80, 81, 83, 84; Selaginelloid Model, 71–73, 77, 81–84
molecular clock, 7, 52
molecular evolution, 1, 4, 7, 264
Monachanthus, 269
Monimiaceae, 360, 362–363
monocotyledons, 27–38, 358, 360–365, 373
monoecious, 233, 266, 269
monopodial branching. *See* branching, monopodial.
monosulcate, 32, 365
Monte Carlo method, 224–226
Moraceae, 148
Mormodes, 269
morphological plasticity, 173–185
mosses, 75, 78–79, 87–95, 101–105; acrocarpous 87, 94–96, 103–105; pleurocarpous, 87, 94–97, 100–103, 105
most recent common ancestor (MRCA), 277, 292–296
multigene families, 47

multilocus gamete, 215, 221, 223–227
multiple copy sequence, 45–47
multiple origins, 305, 321, 360
Musanga, 148
Mutisieae, 370
Myanthus, 269
mycorrhizal fungi, 188
Myristicaceae, 152, 362, 364
Myrtales, xvi

Narcissus, 374
natural selection, 51, 67, 141, 199–200, 204–205, 207–209, 215, 265
nearest neighbor, 21, 30, 32
Nelumbo, 362–363
Neocheiropteris, 120
Nicandra, 157, 159–160, 169
Nicotiana, 158–159, 161–162, 378
Nivenia, 374
Nolanoideae, 158, 160, 165–166
Nordenskioldia, 6
nuclear ribosomal DNA (rDNA), xiv, xvii, 335–336, 340–345, 353, 365
numerical taxonomy, xi, xv
Nuphar, 364
Nyctaginaceae, 357, 368–373
Nymphaeaceae, 362, 364

Ocotea, 363
Odontoschisma, 104
Olacaceae, 373–374
Oleaceae, 374–375
Onagraceae, xi, xiii, xvi–xviii, 357
Onagreae, xvii
Onoclea, 137–138
Ophioglossales, 79, 81
Ophioglossum, 135, 137–139, 142
Opuntioideae, 139
Orchidaceae, 263
orchids, 126, 269
Oreomunnea, 195
Orthotrichum, 103
Osmunda, 137, 140–141
outgroup method, 336
Oxalidaceae, 374–375
paleoherbs, 364–365, 368
Paleozoic, 189
Palustriella, 103
panbiogeography, xvi
Pandananae, 30, 32, 37
Pandanus, 148
panmictic populations, 281, 286, 294, 296
panmixia, 282
Papaveraceae, 357, 360, 362
Papilionoideae, 246, 357
parental effects, 199–200, 206–209
pattern cladists, 320
pattern of diversification, 306
PAUP, 20, 28, 30–33, 336
PCR. *See* polymerase chain reaction.
pearl millet (*Pennisetum glaucum*), 41, 45–47
Pelargonium, 375
Pellia, 90, 98
Pennisetum, 41, 45–53
Pentagramma, 112, 116
Persea, 9, 363
Petalophyllum, 90
Phanerosorus, 123–124
Phaseolus coccineus, 245–257
phenetics, xv
phenotypic plasticity, 67, 104–105, 199–201, 205, 210
Philydrales, 30
Phlox, 266
Phormium, 235
phyllotaxy, 57, 62–66, 91–94, 115, 117, 125–126, 129, 173–174
phylogenetic history, 161, 273–286, 305; phylogenetic systematics, 27–38, 289–302, 305–326, 333–346, 355–376. *See also* species concepts, phylogenetic.
Physalis, 160
phytochrome photoequilibrium, 179, 208
Phytolaccaceae, 370–371
Picea, 157
Piperaceae, 362, 364
Plagiomnium, 103–104
plagiotropy, 112, 115, 120, 122–126, 129–130, 135, 159, 171
plant architecture, viii, 61, 62, 66–67, 147, 157–171
plant demography, xi
Plantago, 207–208
plastome, 345
Platanaceae, 9, 152, 190
Platanocarpus, 191
Platanus, 9, 147, 152, 189–191, 194–195
Platycerium, 129–130, 133–134, 141
Platypodium, 224–225, 228
Pleea, 30
Plumbaginaceae, 374–375
Poaceae, xiv, 27, 29, 37–38, 52, 263, 357
Poales, 29–30, 37–38
Pohlia, 93–94
Polanisia, 370
Polemoniaceae, 374
pollen binucleate, 356, 357, 375, competition, 237–239, 267; exine sculpturing, 268, inaperturate, 269, load, 191, 233–239, 251, triaperturate, 32; tricolpate, 32, 360, 365, 368;

trinucleate, 356–357, 368, 372–375; uniaperturate, 37
pollination: animal, 188–189, 194–195, 202, 218–221; bee, 246; insect, 187–192, 194–195, 224, 227, 267; wind, 189, 193–195, 215–221, 265
Polygalaceae, 368
Polygonaceae, 375
Polygonum, 201–202
polymerase chain reaction (PCR), xiv, xv, 1, 3, 8–10, 19, 312
polyploidy, 309, 314, 321–322, 325
Polypodiaceae, 117
polystichy, 66
Polytrichaceae, 95–96, 101, 103
Pontederiaceae, 373–375
Pontederiales, 30
population differentiation, xiii, 216, 280
population genetics, 53, 273, 278–283, 295, 301
Populus, 6
postzygotic incompatibility, 250
Pottiaceae, 94–96, 103
prezygotic incompatibility, 250
Primulaceae, 374
Proctolaelaps, 46
progeny vigor, 233, 236–239
protoangiosperms, 359
Pseudoscleropodium, 98, 100
Pseudotsuga, 4
Pseudowintera, 363
Psilotaceae, 137
Psilotioid Model. *See* Models of Growth.
Psilotum, 81
Psychotrieae, 372, 375
Ptilium, 97, 100
Punicaceae, 375
Pycnanthus, 147, 152
QTL. *See* quantitative trait locus.
quagaa, 1
quantitative trait locus (QTL), 59–67, 69
Quercus, 9, 299

Racomitrium, 103
radish, 252
Radula, 91, 98
ramets, 174–176, 180–185, 255
random amplified polymorphic DNA (RAPDs), xiv, 15, 19–22, 59, 229
Ranunculaceae, 357–365
Rapateaceae, 30
RAPDs. *See* random amplified polymorphic DNA.
Raphanus, 222, 228, 251
*rbc*L. *See* ribulose 1,5-bisphosphate carboxylase/oxygenase.
rDNA. *See* nuclear ribosomal DNA.
recaulescence. *See* adnation.
reiteration, 75, 82–84, 147–148, 154–157, 161, 169
reproduction, asexual, 121, 291, 296; asexual taxa, 299–300
reproductive biology, viii, xii–xiii, xvi, 113 (habit), 135–139 (structure), 187–195, 199–210, 217, 263–270, 313
reproductive phenology, 201–206
reptiles, 309
Restionaceae, 38
restriction fragment length polymorphisms (RFLPs), 15, 19, 22, 52, 57, 59, 229, 312–313
RETICLAD, 319, 336–344
reticulate evolution, viii, 15, 307, 309, 333–346
reticulation, 275, 291–292, 295, 299–301, 308, 317–318, 321, 333–336, 345–346
retroelements, 48
RFLPs. *See* restriction fragment length polymorphisms.
Rhizobium, 180–181
Rhizomnium, 103
Rhodobryum, 101, 104
Rhynia, 79, 81
ribosomal RNA (rRNA), xiv, 367
ribulose 1,5-bisphosphate carboxylase/oxygenase (*rbc*L), v, xiv–xviii, 1, 3–5, 8, 10, 27–38, 52, 187, 360, 365, 367
Riccardia, 90
Riccia, 88, 90, 98
Racomitrium, 103, 104
Rosaceae, 9, 162, 357
rRNA. *See* ribosomal RNA.
Rubiaceae, 357, 368, 371–376
Rubisco, xiv, 27. *See also* ribulose 1,5-bisphosphate carboxylase/oxygenase (*rbc*L).

Salicaceae, 6
Salmonella, 48
Salpichlaena, 123
Salpiglossidae, 159
Salvinia, 130–132, 135–136
Santalaceae, 373–375
Sapranthus, 363
Saruma, 364
Sassafras, 189
Satyrium, 269
Saururaceae, 362, 364
Saururus, 28, 31
Saxifraga, 120, 122
Saxifragaceae, 32, 357, 374–375
scarlet runner bean (*Phaseolus coccineus*), viii, 246–255, 257
Schisandraceae, 362, 364

Schistidium, 103–104
Schizeaceae, 137
Sciadophyton, 71, 74, 77, 80–81
Scrophulariaceae, 9, 357, 375
Scutellaria, 265, 267
secondary chemistry, 314
secondary sex traits, 65
seed: abortion, 237–239, 255–257; predation, 255; production, 191, 236, 245–257, 357, 363–364
Selaginella, 75
Selaginelloid Model. *See* Models of Growth.
self-compatibility, 59, 234, 266–267, 355–376
self-incompatibility, 22, 59, 246, 250, 266–268, 355–376; gametophytic self-incompatibility, 355–359, 370–375. *See also* sporophytic incompatibility, postzygotic incompatibility, prezygotic incompatibility.
selfing, 16, 19, 22, 59, 190, 202, 215, 217–220, 234–235, 239, 245, 249–250, 256, 265–268, 356–357
selfish DNA, 53
sesquiterpene lactone (SQL), 335, 338, 341–342, 352
Silurian, 73, 188
single gene marker, 215, 222–223, 226
Solanaceae, viii, 157–171, 263, 265, 357, 371, 379
Solanoideae, 158, 160, 162, 165–166, 168
Solanum, 159–161, 266–270
Sonneratiaceae, 375
Southern blot analysis, 49–50, 52
soybean, 252
Sparganium, 29, 189
species: endemic, 217–218; hermaphroditic, 370, 356, 364; heteromorphic, 355–356; homomorphic, 355, 373–374; nature of, 285; tristylous, 373–374 speciation, xiii, 15–16, 46–47, 68, 264, 273, 284, 294, 310, 317, 323–325, 334, 342
species boundaries, 264
species concepts: 285, 289–302; biological, 297, 301; evolutionary, 301; genealogical, 285, 289–302; phylogenetic, 294, 299
species phylogeny, 273, 283–286
species tree, 273, 283–286
Sphagnidae, 89, 92
Sphagnum, 87, 92–93, 98, 105
Splachnum, 94
sporophytic incompatibility, 266, 355–359, 368–374
staghorn fern (*Platycerium*), 129
Stanleya, 370
Stebbinsoseris, 17
Stenochlaena, 112, 115
Sterculiaceae, 162, 357, 368, 372–376
stigmatic inhibition, 356, 372
strict consensus, xvii, 297–298
stylar inhibition, 356
sunflower, 335–336, 338, 344–345, 350, 352
sympodial branching. *See* branching, sympodial.

Tachigali, 223–224, 228
Takakia, 104
Tambourissa, 363
Tasmannia, 363
Taxodiaceae, 3–7, 9
Taxodium, 1, 3–5, 7–10, 27
Telesonix, 375
teosinte, 50–52, 57–69
Tertiary, 6, 194
Tetracentron, 6
Theaceae, 375
Theobroma, 373
Thuidium, 98, 100
Tiarella, 189
Tmesipteris, 81, 137
Tracheophyta, 74, 89
tracheophyte, 78
transantarctic midges, xv
transposable elements (transposons) Activator (*Ac*), 41–45, 48–53, Dissociation (*Ds*), 41–45, 49; horizontal transmission of, 41, 45–48, 53; Mutator (Mu) 42, 44–45; Suppressor, 42, 44
Trichomanes, 121
Trifolium repens, 173–185
Trimeniaceae, 362, 364
Trochodendraceae, 6, 362
Trochodendrales, 6, 362, 364
Trochodendron, 6
tropics, xi
Turneraceae, 374–375

uniformitarian, 305, 310, 322–324
Uropappus, 17
Uskiella, 80

Velloziales, 30
Villarsia, 371, 374
Violaceae, 375

wet stigma, 356
Wettsteinia, 104
Williamsonia, 188
wind dispersal, 137, 187–189, 195, 217, 219, 221
wind pollination. *See* pollination, wind
Winteraceae, 362–363
wolves, 313, 321
Wolffia, 130
Wolffiella, 130

Wright's F_{ST} statistic, 216–221
Wright-Castle formula, 58–59

Zea, 3, 10, 41, 50, 52, 57, 59–61, 57–69.
See also maize.
Zingiberanae, 29–30, 35, 37
Zizyphoides, 6
Zosterophyllum, 80
zucchini, 233–239, 251
Zygogynum, 363

Missouri Botanical Garden Library
3 1753 00201 1861

ISBN 0-915279-30-4